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Editorials

Does stress cause cancer?

There's no good evidence of a relation between stressful events and cancer

Papers p 1027

In 1893 Snow presented what might be the first statistical summary of the psychological characteristics of patients with breast or uterine cancer.[1] Some 250 women with these cancers were described as having a "general liability to the buffets of ill-fortune." Over 100 years later we still find researchers preoccupied with showing whether stressful life events are related to cancer—as in this week's study by Protheroe et al (p 1027).[2] Many clearly believe that life is more stressful than ever before and that one consequence of this ubiquitous stress is disease, including cancer. Sontag describes this as a metaphorical view of disease as the "outward expression of character."[3] In more practical terms, patients with cancer may believe that their disease results from too much stress and relatives may feel guilty for contributing to the emotional ill health of the patient. Such beliefs may also have a bearing on what people do about seeking and sticking to treatment. It is important therefore to have a clear idea of what the evidence does show.

Two recent literature reviews have concluded that there is no good evidence for any relation between stressful life events and breast cancer,[4,5] and both point out that the typical methods used in studies of the relation are problematic at best. What then should we make of this most recent study? The methods used are fairly well in line with previous research.[2] Women attending breast clinics in west Leeds after discovering a suspicious breast lump but before learning the outcome of biopsy were asked about life stresses in the previous five years. Biopsy outcome then identified those with malignancy (106) and those with benign disease (226). Women with malignancy were no more likely to experience one or more severe life events (adjusted odds ratio 0.91) or severe difficulties (odds ratio 0.86) in the previous five years than those with a benign lump.

While consistent with the recent literature reviews, these findings stand in contrast to an earlier report by Chen et al, in the *BMJ*, using much the same methods, which suggested that women with breast cancer were nearly 12 times more likely to experience severe life events over the same period before diagnosis.[6] Why the discrepancy and what do these findings tell us about the relation between life events and breast cancer?

It is arguable whether the methods used in either of these studies could ever represent an adequate test of the hypothesis of a link between stress and cancer. Retrospective recall of life events in the five years before learning whether a breast lesion is malignant or benign constitutes a relatively weak test of the hypothesis, compared with good prospective studies. In Protheroe et al's study, even this most basic safeguard against recall bias was ignored as 30% of the women with cancer knew their diagnosis by the time they were interviewed.[2]

Two other features of the two studies are worth comment. Both are described as case-control studies but might be better described as cross sectional. In a true case-control study the controls are drawn from the same population as the cases. However, the women with cancer are considerably older—an average of 10.6 years in the study of Protheroe et al and 7 years in that of Chen et al. Many of the other studies using these methods report similar age differences.[5] It is unclear to what extent these studies can control for such large age differences in their analyses. This is important as age relates directly to risk of breast cancer and to experiencing particular life events.[5]

Both studies also use multivariate modelling with many predictors and relatively few outcome events —that is, cancers. A general rule of thumb is that there should be at least 10 outcome events for each predictor entered into the model,[7] so the multivariate analyses in these studies are probably overfitted and the estimates unstable. This is particularly so in the study of Chen et al, where12 predictors were entered into a model based on 41 women with cancer. While adjustment for other factors should lead to more precise estimates of effects, the reverse is true in their analysis, with the unadjusted odds ratio increasing from about 3 to 12 in the adjusted model, with a correspondingly large increase in the confidence interval surrounding the estimate. This suggests that life events are so correlated with one or more of the other variables that it is difficult to disentangle their effect. Similar criticisms apply to Protheroe et al's study, with 19 predictors entered into their model.

It is easy to go on picking holes in the methods of these types of studies—and perhaps unfair. One difficulty is that the hypothesis being tested is so vague. This is not the fault of the authors; the literature has not developed much beyond such vagueness. Any hypothesised relation does not seem to relate to cancer causation (causative factors may well be operating many years before detection) but may have something to do with stress accelerating the development of lesions or otherwise influencing the probability of diagnosis. The hypothesis needs to be stated in some more biologically plausible form to allow a stronger test of the association. Prospective longitudinal designs would be a good place to start.

Already some indications exist from prospective studies that there is no relation between stressful events and cancer. The results of a large scale study in the United Kingdom provide little evidence for an association between bereavement in men or women and later cancer.[8] Other research has investigated the long term outcome for prisoners during the second world war and the Korean war.[9] These men clearly suffered extremes of physical and mental hardship, and though they showed excess mortality due to accidental injury, suicide, and cirrhosis of the liver—suggesting continuing psychological distress —there was no excess mortality due to cancer. A second longitudinal study of Japanese men living in Hawaii showed no relation between stressful life situations and later cancer.[10]

Recriminations over real or imagined life stress may be counterproductive for individuals with cancer

and their families. They should be reassured that the available scientific evidence does not support any direct role for stressful life events leading to a diagnosis of cancer.

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1. Snow HL. *Cancer and the cancer process.* London: Churchill, 1893.
2. Protheroe D, Turvey K, Horgan K, Benson E, Bowers D, House A. Stressful life events and onset of breast cancer: case-control study. *BMJ* 1999; 319: 1027-1030[Abstract/Full Text].
3. Sontag S. *Illness as metaphor.* London: Penguin, 1979.
4. Petticrew M, Fraser JM, Regan MF. Adverse life events and risk of breast cancer: a meta analysis. *Br J Health Psychol* 1999; 4: 1-17.
5. McGee R, Williams S, Elwood M. Are life events related to the onset of breast cancer? *Psychol Med* 1996; 26: 441-447[Medline].
6. Chen CC, David AS, Nunnerley H, Michell M, Dawson JL, Berry H, et al. Adverse life events and breast cancer: case-control study. *BMJ* 1995; 311: 1527-1530[Abstract/Full Text].
7. Harrell FE, Lee KL, Mark DB. Tutorial in statistics. Multivariable prognostic models: issues in developing models, evaluating assumptions and adequacy, and measuring and reducing errors. *Statist Med* 1996; 15: 361-387[Medline].
8. Jones DR, Goldblatt PO, Leon DA. Bereavement and cancer: some data on deaths of spouses from the longitudinal study of Office of Population Censuses and Surveys. *BMJ* 1984; 289: 461-464 [Medline].
9. Keehn RJ. Follow up studies of World War II and Korean conflict prisoners: III. Mortality to January 1, 1976. *Am J Epidemiol* 1980; 111: 194-211[Medline].
10. Joffress M, Reed DM, Nomura AMY. Psychosocial processes and cancer incidence among Japanese men in Hawaii. *Am J Epidemiol* 1985; 121: 488-500[Medline].

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PAPERS

Stressful life events and difficulties and onset of breast cancer: case-control study.

David Protheroe, Kim Turvey, Kieran Horgan, Eddie Benson, David Bowers, and Allan House
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Psychological Stress and Cancer

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CANCER FACTS National Cancer Institute National Institutes of Health The complex relationship between physical and psychological health is not well understood. Scientists know that many types of stress activate the body's endocrine (hormone) system, which in turn can cause changes in the immune system, the body's defense against infection and disease (including cancer). However, the immune system is a highly specialized network whose activity is affected not only by stress but by a number of other factors. It has not been shown that stress-induced changes in the immune system directly cause cancer.

Some studies have indicated an increased incidence of early death, including cancer death, among people who have experienced the recent loss of a spouse or other loved one. However, most cancers have been developing for many years and are diagnosed only after they have been growing in the body for a long time (from 2 to 30 years). This fact argues against an association between the death of a loved one and the triggering of cancer.

The relationship between breast cancer and stress has received particular attention. Some studies of women with breast cancer have shown significantly higher rates of this disease among those women who experienced traumatic life events and losses within several years before their diagnosis. Although studies have shown that stress factors (such as death of a spouse, social isolation, and medical school examinations) alter the way the immune system functions, they have not provided scientific evidence of a direct cause-and-effect relationship between these immune system changes and the development of cancer. One NCI-sponsored study suggests that there is no important association between stressful life events, such as the death of a loved one or divorce, and breast cancer risk. However, more research to find if there is a relationship between psychological stress and the transformation of normal cells into cancerous cells is needed.

One area that is currently being studied is the effect of stress on women already diagnosed with breast cancer. These studies are looking at whether stress reduction can improve the immune response and possibly slow cancer progression. Researchers are doing this by determining whether women with breast cancer who are in support groups have better survival rates than those not in support groups.

Many factors come into play when determining the relationship between stress and cancer. At present, the relationship between psychological stress and cancer occurrence or progression has not been scientifically proven. However, stress reduction is of benefit for many other health reasons.

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National Cancer Institute Information Resources

You may want more information for yourself, your family, and your doctor. The following National Cancer Institute (NCI) services are available to help you.

Telephone...

Cancer Information Service (CIS)

Provides accurate, up-to-date information on cancer to patients and their families, health professionals, and the general public. Information specialists translate the latest scientific information into understandable language and respond in English, Spanish, or on TTY equipment.

Toll-free: 1-800-4-CANCER (1-800-422-6237)
TTY: 1-800-332-8615

Internet...

These web sites may be useful:

Http: //www.nci.nih.gov - NCI's primary web site; contains information about the Institute and its programs.

Http: //cancernet.nci.nih.gov - CancerNet; contains material for health professionals, patients, and the public, including information from PDQ about cancer treatment, screening, prevention, genetics, supportive care, and clinical trials, and CANCERLIT, a bibliographic database.

Http: //cancertrials.nci.nih.gov - cancerTrials; NCI's comprehensive clinical trials information center for patients, health professionals, and the public. Includes information on understanding trials, deciding whether to participate in trials, finding specific trials, plus research news and other resources.

E-mail...

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Stress and relapse of breast cancer

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Abstract

To elucidate the association between stressful life events and the development of cancer the influence of life stress on relapse in operable breast cancer was examined in matched pairs of women in a case-control study. Adverse life events and difficulties occurring during the postoperative disease free interval were recorded in 50 women who had developed their first recurrence of operable breast cancer and during equivalent follow up times in 50 women with operable breast cancer in remission. The cases and controls were matched for the main physical and pathological factors known to be prognostic in breast cancer and sociodemographic variables that influence the frequency of life events and difficulties. Severely threatening life events and difficulties were significantly associated with the first recurrence of breast cancer. The relative risk of relapse associated with severe life events was 5·67 (95% confidence interval 1·57 to 37·20), and the relative risk associated with severe difficulties was 4·75 (1·58 to 19·20). Life events and difficulties not rated as severe were not related to relapse. Experiencing a non-severe life event was associated with a relative risk of 2·0 (0·62 to 7·47), and experiencing a non-severe difficulty was associated with a relative risk of 1·13 (0·38 to 3·35).

These results suggest a prognostic association between severe life stressors and recurrence of breast cancer, but a larger prospective study is needed for confirmation.

Introduction

The association between stressful life events and the development of cancer is supported by a large body of anecdotal clinical evidence that has been collected since the eighteenth century. Recent, more substantive clinical and epidemiological evidence for a link between life events and the onset of cancer has been less consistent and flawed by conceptual and methodological weaknesses.[1,2] The most important of these shortcomings has been the inability to date accurately the onset of tumour growth. This is a necessary prerequisite for any study claiming to investigate the influence of stress on that onset. Moreover, unreliable measures of stressful experiences have been used and control groups have been poorly chosen.

The role of life events in the prognosis of cancer has received much less attention but is more easily studied because progression of the disease is more amenable to measurement. Early and accurate diagnosis of relapse is facilitated by the regular follow up of patients with cancer in oncology units. Using a measure of life events that overcomes many of the problems of low reliability and validity that have undermined research on life stress,[3] we examined the influence of adverse life experiences on the development of relapse in women with operable breast cancer.

Patients and methods

Data on life events were collected from 50 consecutive women who had developed a first recurrence after treatment of operable breast cancer. Recurrences (local or distant) were diagnosed according to the criteria of Hayward *et al.*[4] Similar data were obtained from a control group of women whose operable breast cancer was in remission according to clinical and investigatory criteria.

The matching of the women who had a relapse with their controls was performed by computer searches of the database at the clinical oncology unit, Guy's Hospital, which contains clinical, pathological, and demographic information on all women with breast cancer who have attended the unit. The cases and controls were matched in pairs for the main physical and pathological factors known to be prognostic in breast cancer. These included type of operation, whether or not the patient had received adjuvant chemotherapy, menopausal state, affected lymph nodes, tumour size, and histological type of tumour. The cases and controls were then also matched for date of operation and those sociodemographic variables that influence the frequency of life events in the general population.

For the women whose breast cancer relapsed the life events data were collected for the period between the date of operation and the date of recurrence. The life events data for the controls were ascertained over the equivalent follow up period from the date of their operation. Adverse life experiences were measured with the Bedford College life events and difficulties schedule,[5] an instrument based on an interview that assesses not only discrete life events but also more persistent, continuing difficulties. Only those adverse life events and difficulties (stressors) that met strictly predefined criteria were considered for inclusion. The severity of stressors was rated by a panel of judges according to their undesirability and threat to the subject. Ratings were based on how a hypothetical woman would be expected to react, given the details of the events, the circumstances surrounding them, and the woman's biography. The raters were kept ignorant of the subject's emotional reaction to the event. This approach reduced any bias stemming from the subject, whose recall and report of events may have been influenced by an attempt to make sense of her disease. Because the ratings were made by a panel of independent judges the potential bias stemming from the investigator was brought under control. Raters did not know whether the event was followed by a recurrence of disease, thus avoiding the influence of any judgments about likely causal links between events and relapse of the disease.

Life events were rated as severe if they had threatening implications in the long term and consequences that were either pronounced or moderate and focused on the woman herself or jointly with someone else, such as the death of a husband or child, divorce, or arguments leading to a complete breakdown of important family relationships. Difficulties that carried a pronounced threat and persisted for at least six months were similarly rated as severe—for example, the problem for a 52 year old woman of caring for her 22 year old son, severely physically handicapped with cerebral palsy, as she provided 24 hour

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total care for him. Life events and difficulties rated as not severe were those that carried only little or no long term threat: events such as the only son of a happily married woman leaving home to join the army and being expected to do tours in Northern Ireland, and difficulties such as the husband who drinks three to four pints of beer a night and spends most of the extra family money on alcohol, who may be quarrelsome but is never violent and has always been in employment.

The relative risk of relapse associated with life events and difficulties was calculated as the Mantel-Haenszel odds ratio, and 95% confidence intervals were derived by the exact method. Values for χ^2 and p were derived from McNemar's test with continuity correction for the pair matched case-control data.[5] These analyses were based on pairs of women who were discordant for the experience of a stressor—namely, those pairs in which one member had a stressor and the other did not. The concordant pairs, in which both or neither experienced a stressor, did not contribute any evidence for causality and so were ignored in the analyses.

Results

All the women who were asked to participate in the study agreed to be interviewed. The average age of the women was 49·5 (SD 9·2) years. Altogether 83 were married, three were single, four were divorced, and 10 were widowed. The median interval free of disease for the women who had a relapse of breast cancer was 30·5 months (range 9-145).

TABLE I—*Matching of 50 women who had a relapse of breast cancer (cases) and 50 women who did not (controls) for physical and pathological factors*

	No of cases	No of controls
Primary operation:		
Mastectomy	36	36
Conservation procedure	14	14
Adjuvant chemotherapy:		
None	38	38
Melphalan	3	3
Cyclophosphamide, methotrexate, fluorouracil	9	9
Menopausal state:		
Premenopausal	29	28
Perimenopausal	6	6
Postmenopausal	15	16
No of axillary lymph nodes affected:		
0	19	19
1-3	19	17
4-15	11	13
>15	1	1
Histological type of tumour:		
Ductal grade I	2	2
Ductal grade II	23	23
Ductal grade III	19	20
Lobular	5	4
Medullary	1	0
Tumour size:		
T_1	12	10
T_2	32	37
T_3	6	3

The pairs were well matched for the treatments they received, with only minor mismatches for menopausal state, number of affected axillary lymph nodes, tumour size, and histological type of tumour. There were no major mismatches for age, marital state, social class, and life stage (an index reflecting age and the presence of children in the subject's household). Table I gives an overall comparison of the prognostic factors in the cases and controls.

Table II shows the numbers of pairs of women who experienced at least one adverse stressor during the disease free interval or the equivalent follow up time. In 10 pairs only the woman who had a relapse had experienced any adverse life event and in four pairs only the control had. Hence the relative risk for relapse associated with an adverse life event of any severity was 2·5 (p=0·2). Experience of non-severe life events was associated with a lower relative risk of 2·00 (p=0·3). In 17 pairs the woman who had a relapse had experienced a severe life event and her control had not, and in three pairs the control had experienced a severe life event and the woman who had a relapse had not. Thus, a significant relative risk (5·67) was associated with the experience of a severe life event (p=0·004).

The relative risk of relapse associated with the experience of difficulties followed a similar pattern. For difficulties of any severity the relative risk was 2·80 (p=0·7) and for non-severe difficulties 1·13 (p=1·0), whereas experience of severe difficulties was associated with a significant relative risk of 4·75 (p=0·004). Experiencing either a severe life event or a severe difficulty was associated with an even greater relative risk (9·00): there were nine times as many pairs in which only the woman who had a relapse had experienced a severe stressor of either type as there were pairs in which only the control had (p<0·001).

Discussion

The findings of this study suggest a prognostic association between severe life stressors and recurrence of operable breast cancer. Whether overall survival from breast cancer is altered by severe stress has yet to be determined. Results of recent studies using cancer mortality statistics provide some support for the link between adverse life events and survival with cancer. Based on data from the Office of Population Censuses and Surveys a weak association was found between death of a wife and subsequent death from cancer of the widower after a long latent interval,[7] but further follow up of the cohort of widowers is required before final conclusions can be drawn. Also, decreased survival with breast cancer was shown in women aged over 60 who had experienced death, illness, or unemployment in members of their household in the five years before their malignant disease was diagnosed.[8]

The design of our study overcame many of the

TABLE II—*Relation between life stressors and relapse in 50 pairs of women with breast cancer*

	No of concordant pairs		No of discordant pairs		Relative risk of relapse associated with experience of stressor		
Type of stressor	Both patients experienced stressor	Neither patient experienced stressor	Only patient who relapsed experienced stressor	Only control experienced stressor	Odds ratio* (95% confidence interval)	χ^2†	p†
Any event	34	2	10	4	2·50 (0·72 to 10·93)	1·79	0·181
Non-severe event	31	4	10	5	2·00 (0·62 to 7·47)	1·07	0·303
Severe event	9	21	17	3	5·67 (1·57 to 37·20)	8·45	0·004
Any difficulty	21	10	14	5	2·80 (0·95 to 9·93)	3·37	0·066
Non-severe difficulty	14	19	9	8	1·13 (0·38 to 3·35)	0·00	1·000
Severe difficulty	6	21	19	4	4·75 (1·58 to 19·20)	8·52	0·004
Any severe stressors‡	13	17	18	2	9·00 (2·15 to 79·97)	11·25	<0·001

objections of the previous studies that looked at relation and the development of cancer. In particular, we rigorously controlled for the main physical and pathological factors that are known to influence prognosis in breast cancer. A measure of life stressors was used that accurately dated events and difficulties to ensure that they preceded the clinical onset of progression of the disease. This measure also attempted to assess the objective threat of life events and difficulties independently of both the subject's emotional reaction and investigator bias. This is important as neither the patient nor the investigator could be blinded to the woman's disease state. Unconsciously motivated differences in the subject's recall and interviewer's techniques remain a possible source of bias. If these were operating, however, a more systematic excess of both non-severe and severe stressors among the women with a relapse would be expected.

The small numbers of women who participated in this study must be borne in mind when interpreting the results, a factor that is reflected in the wide confidence intervals associated with the estimates of relative risk. The findings of this study now need to be corroborated in a large prospective investigation.

The mechanism whereby stress might affect the relapse of breast cancer is unknown. Suggested intermediaries include the neuroendocrine[9] and immune systems,[10 11] which could promote growth of previously dormant or subclinical metastases. Investigations of this are complex and difficult.[12 13] Modifications in behaviour leading to direct exposure to carcinogens must also be considered as a possible mediating process.

The impact of severe life stressors on the recurrence of breast cancer may be modified by other psychosocial factors. Further analysis is required to explore the interaction between severe life stressors and variables such as coping behaviour and social support, both of which have been suggested as prognostic factors in themselves.[15-17] Understanding the nature of such important implications for management of patients and for the development of cognitive[18 1] and other psychological treatments aimed at helping patients with cancer adjust to the impact of their disease and cope with the consequences of subsequent severe life stressors.

1 Ramirez AJ. Life events and cancer: conceptual and methodological issues. In: Watson M, Greer S, Thomas C, eds. *Psychosocial oncology*. Oxford: Pergamon, 1988:53-60.
2 Craig TKJ, Brown GW. Life events: meaning and physical illness. In: Steptoe A, Mathews A, eds. *Health care and human behaviour*. New York: Academic Press, 1984:7-39.
3 Brown GW, Harris T. *Social origins of depression*. Cambridge: Cambridge University Press, 1978.
4 Hayward JL, Meakin JM, Stewart HJ. Assessment of response and recurrence in breast cancer. *Sem Oncol* 1978;4:445-9.
5 Breslow NE, Day NE. *Statistical methods in cancer research*. Vol 1. *The analysis of case control studies*. Lyons: International Agency for Research on Cancer, 1980.
6 Ashraf J, Fentiman IS. Conservative treatment of early breast cancer. *Br J Hosp Med* 1986;35:263-5.
7 Jones DR, Goldblatt PO, Leon DA. Bereavement and cancer: some data on deaths of spouses from the longitudinal study of Office of Population Censuses and Surveys. *Br Med J* 1984;289:461-4.
8 Funch DP, Marshall J. The role of stress, social support and age and survival from breast cancer. *J Psychosom Res* 1983;27:77-83.
9 Katz J, Gallagher TS. Psychoendocrine considerations in cancer of the breast. *Ann NY Acad Sci* 1969;164:509-16.
10 Kiecolt-Glaser JN, Garner W, Speicher C, Penn GM, Holloway BS. Psychosocial modifiers of immunocompetence in medical students. *J Psychosom Med* 1984;46:7-14.
11 Bartrop RW, Lazarus L, Luckhurst E, Kiloh LG, Penny R. Depressed lymphocyte function after bereavement. *Lancet* 1977;i:834-6.
12 Fox BH. Psychosocial factors in the immune system in human cancer. In: Ader R, ed. *Psychoimmunology*. New York: Academic Press, 1981:103-58.
13 Cox T, Mackay C. Psychosocial factors and psychophysiological mechanisms in the aetiology and development of cancer. *Soc Sci Med* 1982;16:381-96.
14 Pettingale KW. Towards a psychobiological model of cancer: biological considerations. *Soc Sci Med* 1985;20:179-87.
15 Pettingale KW, Morris T, Greer S, Haybrittle JL. Mental attitudes to cancer: an additional prognostic factor. *Lancet* 1981;i:750.
16 Temoshok L. Biopsychological studies in cutaneous malignant melanoma. *Soc Sci Med* 1985;20:833-40.
17 Goodwin JS, Williams WC, Key CR, Sarnet JM. The effect of marital status on stage, treatment and survival of cancer patients. *JAMA* 1987;258:3125-30.
18 Greer S, Moorey S. Adjuvant psychological therapy for cancer patients. *Eur J Surg Oncol* 1987;13:511-16.
19 Tarrier N, Maguire P, Kincey J. Locus of control and cognitive behaviour therapy with mastectomy patients. *Br J Med Psychol* 1983;56:265-70.

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Smoking in hospitals: a measure of improvement

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The argument for restrictions on smoking in health service premises is clear. Most smokers and non-smokers support restrictions in hospitals,[1] and the Department of Health and Social Security set out firmly the exemplary role that health authorities should have in the campaign to stop smoking.[2]

A survey of all health service premises in the Wessex region was undertaken in 1981, and the information obtained was used to set goals for reducing smoking in hospitals and health centres by 1985.[3 4] We carried out a study in 1985 to monitor the progress towards these goals and to identify improvements that might be made.

Methods and results

We asked administrators of all health service premises in Wessex to complete a postal questionnaire identical with that used in the survey in 1981.[3] We requested information on the extent of restrictions on smoking in their premises; how restrictions on smoking were identified and monitored; and sales of validate the results. Replies were received from 246 of the 250 premises.

RESTRICTIONS ON SMOKING IN PUBLIC AREAS

Short stay hospitals (n=61)—The proportion of hospitals achieving the recommended goals in wards and outpatient and other public areas had increased since 1981 (table). Only 23 of the hospitals, however, had achieved the desired goal for day rooms. Fifty three offered day rooms where smoking was permitted, while only 26 provided day rooms that were always smoke free.

Maternity hospitals (n=7)—The high level of restrictions found in 1981 was maintained, with no smoking in any of the wards. Restrictions in day rooms were less satisfactory.

Psychiatric hospitals (n=32)—Low levels of restrictions were found in all areas.

Long stay and geriatric hospitals (n=45)—The proportion of premises with satisfactory restrictions had increased since 1981.

Health centres (n=44)—Forty two of the health centres had a total ban on smoking in public areas.

The task of monitoring restrictions was undertaken by nurses and administrators, while doctors had a minor role.

RESTRICTIONS ON SMOKING IN STAFF AREAS

Canteen and restaurant facilities—Restrictions had

the infants were at high risk of the HBV carrier state but the effects of breastfeeding on mother-to-infant HBV transmission were not studied.[16] In our study serum from all ten HBeAg-positive, HBsAg-carrier mothers contained HBV DNA; HBV DNA was not detected in the single HBeAg-negative, HBsAg-carrier mother. HBV DNA was found in only one cord blood sample. No HBV DNA was found in the infant's serum after the administration of HBIg, for as long as moderate levels of anti-HBs persisted. As anti-HBs disappeared, both HBV DNA and HBsAg were detected in the serum (9 months of age) despite four doses of hepatitis B vaccine. These findings suggested at least three possible causes of vaccination failure. The first possibility is that HBV infection of the fetus in utero made the child immunologically tolerant to HBV antigens, so that HB vaccine was not effective. Secondly, early administration of HBIg could have protected the child from viraemia, but HBV had already infected leucocytes, liver cells, or other cells and was reactivated later. The third possibility is that the baby was genetically a low responder to the epitopes of the vaccine antigens and was horizontally infected. The induction of immunological tolerance by intrauterine infection could explain the vaccine failure in this baby. To explore the second possibility leucocytes from the infant would have to be examined during the perinatal period. Shen and colleagues[5] described two cases of HBV DNA detected in cord blood leucocytes. Non-responsiveness to HBsAg is associated with genes in the HLA DR regions.[17] The third possibility may explain the fact that the baby's sister was also a low responder to hepatitis B vaccine.

These findings suggest the need for a follow-up study of whether breastfeeding by HBV-carrier mothers is advisable.

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REFERENCES

1. Beasley RP, Hwang LY, Lee GC, Lan CC, Roan CH, Huang FY. Prevention of perinatally transmitted hepatitis B virus infections with hepatitis immune globulin and hepatitis vaccine. *Lancet* 1983; ii: 1099–102.
2. Yano M, Tajima H. Prevention of HBV mother-infant transmission. *Acta Pediatr Jpn* 1986; 28: 300–05.
3. Bonino F, Hoyer B, Nelson J, Engle R, Verme G, Gerin J. Hepatitis B virus DNA in sera of HBsAg carriers: a marker of active hepatitis B virus replication in the liver. *Hepatology* 1981; 1: 386–91.
4. Shen HD, Choo KB, Lee SD, Tsai YT, Han SH. Hepatitis B virus DNA in leukocytes of patient with hepatitis B virus-associated liver disease. *J Med Virol* 1986; 18: 210–11.
5. Shen HD, Choo KB, Wu TC, Ng HT, Han SH. Hepatitis B virus infection of cord blood leukocytes. *J Med Virol* 1987; 22: 211–16.
6. Jenison SA, Lemon SM, Baker LN, Newbold JE. Quantitive analysis of hepatitis B virus DNA in saliva and semen of chronically infected homosexual men. *J Infect Dis* 1987; 156: 299–307.
7. Hadchouel M, Pasquinelli C, Fournier JG, et al. Detection of mononuclear cells expressing hepatitis B virus in peripheral blood from HBsAg positive and negative patient by in situ hybridization. *J Med Virol* 1988; 24: 27–32.
8. Kaneko S, Miller RH, Feinstone SM, et al. Detection of serum hepatitis B virus DNA in patients with chronic hepatitis using the polymerase chain reaction assay. *Proc Natl Acad Sci USA* 1989; 86: 312–16.
9. Imai M, Yamashita Y, Miyakawa M, Mayumi M. Haemagglutination inhibition assay of the common determinant and subspecificities of Australia antigen. *Immunology* 1974; 27: 871–78.
10. Tsuda F, Takahashi T, Takahashi K, Miyakawa Y, Mayumi M. Determination of antibody to hepatitis B core antigen by means of immune adherence hemagglutination. *J Immunol* 1975; 115: 834–38

References continued at foot of next column

EFFECT OF PSYCHOSOCIAL TREATMENT ON SURVIVAL OF PATIENTS WITH METASTATIC BREAST CANCER

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Summary The effect of psychosocial intervention on time of survival of 86 patients with metastatic breast cancer was studied prospectively. The 1 year intervention consisted of weekly supportive group therapy with self-hypnosis for pain. Both the treatment (n = 50) and control groups (n = 36) had routine oncological care. At 10 year follow-up, only 3 of the patients were alive, and death records were obtained for the other 83. Survival from time of randomisation and onset of intervention was a mean 36·6 (SD 37·6) months in the intervention group compared with 18·9 (10·8) months in the control group, a significant difference. Survival plots indicated that divergence in survival began at 20 months after entry, or 8 months after intervention ended.

Introduction

MANY studies have demonstrated positive psychosocial effects of group therapy in cancer patients, including improvements in mood, adjustment, and pain.[1-4] However, few studies have prospectively examined medical effects.[5-9] In general, patients who receive psychotherapy survived longer. Our objective was to assess whether group therapy in patients with metastatic breast cancer had any effect on survival. This group intervention has been reported[10] to improve the psychological well-being of such patients. We started with the belief that positive psychological and symptomatic effects could occur without affecting the course of the disease; we expected to improve the quality of life without affecting its quantity. Here we describe a 10 year follow-up of the effect of psychosocial intervention on disease progression and mortality.

Patients and Methods

Patients

Only subjects with documented metastatic carcinoma of the breast were included. 109 women were referred by their oncologists. Those patients who agreed were called upon by our research interviewer, who told them about the study and invited

T. MITSUDA AND OTHERS: REFERENCES—*continued*

11. Davis LG, Dibner MD, Battey JF. Basic methods in molecular biology. New York: Elsevier, 1986: 44–46.
12. Fujiyama A, Miyahara A, Nozaki C, Yoneyama T, Ohtomo N, Matsubara K. Cloning and structure analysis of hepatitis B virus DNAs, subtype *Adw*. *Nucl Acids Res* 1983; 11: 4601–10.
13. Saiki RK, Gelfand DH, Stoffel S, et al. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science* 1988; 239: 487–91.
14. Beasley RP, Stevens CE, Shiao IS, Meng HC. Evidence against breast-feeding as a mechanism for vertical transmission of hepatitis B. *Lancet* 1975; ii: 740–41.
15. Lee AKY, Ip HMH, Wong VCW. Mechanisms of maternal-fetal transmission of hepatitis B virus. *J Infect Dis* 1978; 138: 668–71.
16. Ip HMH, Lelie PN, Wong VCW, Kuhns MC. Prevention of hepatitis B virus carrier state in infants according to maternal serum levels of HBV DNA. *Lancet* 1989; i: 406–09.
17. Craven DE, Awdeh ZL, Kunches LM, et al. Nonresponsiveness to hepatitis B vaccine in health care workers. *Ann Intern Med* 1986; 105: 356–60.

TABLE I—DETAILS OF CONTROL AND INTERVENTION PATIENTS

—	Control (n=36)	Intervention (n=50)
Age		
At initial diagnosis	49·3 (10·5)	49·9 (10·0)
At study entry	54·6 (10·2)	54·7 (9·9)
Married	25 (69%)	26 (52%)
Type of surgery		
Lumpectomy	6 (17%)	3 (6%)
Simple mastectomy	2 (6%)	2 (4%)
Modified radical mastectomy	11 (31%)	18 (36%)
Radical mastectomy	17 (47%)	26 (52%)
*Degree of metastatic spread**		
Soft tissue	0·3 (0·5)	0·5 (0·6)
Viscera	0·3 (0·5)	0·3 (0·5)
Bone	0·4 (0·7)	0·3 (0·7)
Initial stage†		
I	3 (8%)	8 (16%)
II	18 (50%)	16 (32%)
III	5 (14%)	11 (22%)
IV	7 (19%)	2 (4%)
No of mastectomies‡	1	1
Exercise		
Hours per week	1·7 (0·8)	1·6 (0·8)
Activity level§	1·8 (1·1)	1·8 (1·1)
No of treatment courses at entry‡		
Chemotherapy	1	1 (0, 1)
Oestrogen	0 (0, 1)	0
Androgen	0 (0, 1)	0
Steroid	0	0
Irradiation	1 (0, 1)	1 (0, 1)
Days of irradiation	41·7 (81·9)	18·8 (24·8)

Mean (SD) or no of cases.
*Spread of metastasis scaled as: 0 = no spread, 1 = one site, 2 = more than one site of a particular type, and 3 (bone only) = four or more sites.
†χ^2 for trend, $p < 0.07$.
‡Median (lower and upper quartiles = median except where indicated).
§Self-rating 1–5.

them to participate. Of this group, 86 completed the first questionnaire, while 18 others refused to participate and 5 died before contact. After written informed consent was obtained (protocol approved by Stanford Human Subjects Committee), a battery of psychological tests was administered. The subjects were then randomly assigned to either the intervention or control groups, and initial follow-up was done every 4 months for a year. More subjects were randomly assigned to therapy (n = 50) than to control (n = 36) to ensure enough patients for group work. 14 subjects assigned to group therapy were too weak or ill at initial interview to participate; 6 died after entry but before the groups began and 2 others moved away. 12 subjects were lost from the controls; 4 were too ill to participate, 2 died, 4 refused to participate, and we were unable to contact 2. 30 of the 34 women in the intervention group and all 24 control women survived long enough to respond to at least one follow-up questionnaire during the year of study. During the first year there was no indication of improved survival in the treatment group; in fact, slightly more patients in this group died during this period (30 *vs* 22%, χ^2 not significant).

Survival time, obtained for all patients who entered the study, was based on state death records for 83 patients. All but 2 of these had breast cancer listed as the immediate or contributing cause of death. The 2 deaths not related to cancer occurred in the controls: 1 cerebrovascular accident and 1 suicide. We made phone contact with the 3 survivors. For other than the primary survival analysis, these 3 were treated as though their date of death was July 1, 1988, when all death records had been obtained. If there was any bias resulting from this decision, it would be in the direction of minimising the impact of intervention, since all 3 were in the treatment group.

The two groups were similar at study entry except for a nearly significant difference in staging at initial diagnosis (tables I and II). Staging information, based on medical records at study entry, was available for 70 of the 86 patients. Initial staging favoured the intervention group. Initial staging took place, on average, 59·8 (SD 47·6) months before the beginning of the study. Patients were not referred to us until they had metastatic disease. Since some studies show that staging is a predictor of survival,[11–13] it could be that by chance the treatment sample had a better prognosis when initially diagnosed than the controls. We found, however, that initial staging was unrelated to survival from the time of randomisation until death. Nonetheless, staging was a control variable during analysis.

TABLE II—DISEASE COURSE PRE-ENTRY (MONTHS)

—	Control	Intervention
Initial diagnosis to first metastasis	38·0 (44·9)	36·3 (35·6)
First metastasis to entry	24·4 (17·4)	21·9 (21·8)
Initial diagnosis to entry	62·3 (53·5)	58·0 (43·3)

Mean (SD).

Intervention

The intervention lasted for a year while both control and treatment groups received their routine oncological care. The three intervention groups met weekly for 90 min, led by a psychiatrist or social worker with a therapist who had breast cancer in remission. The groups were structured to encourage discussion of how to cope with cancer, but at no time were patients led to believe that participation would affect the course of disease. Group therapy patients were encouraged to come regularly and express their feelings about the illness and its effect on their lives. Physical problems, including side-effects of chemotherapy or radiotherapy, were discussed and a self-hypnosis strategy was taught for pain control.[14] Social isolation was countered by developing strong relations among members. Members encouraged one another to be more assertive with doctors. Patients focused on how to extract meaning from tragedy by using their experience to help other patients and their families. One major function of the leaders was to keep the groups directed toward facing and grieving losses.[10]

Analysis

The analysis used Cox's proportional hazards model to examine whether intervention affected survival. This model was chosen so that we could assess the influence of treatment assignment over and above the effect of pre-randomisation prognostic variables by O'Brien's logit-rank procedure.[15] The log-rank test was also used to ensure that main effect differences were significant although the hazards of survival differed. We also drew Kaplan-Meier plots, and used unpaired t, Wilcoxon's rank sum, and χ^2 tests where appropriate.

Results

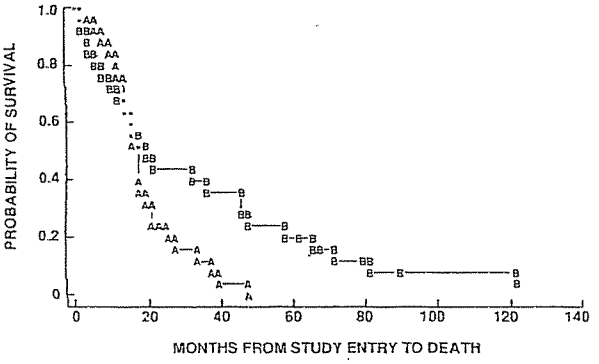
Most striking was the difference in survival from time of randomisation, when intervention began, until date of death. Survival time for the treatment group was significantly longer compared with controls (table III and figure). In addition the interval from first metastasis to death was significantly longer for the group randomised to treatment. Thus the intervention group lived on average twice as long as did controls.

Since initial staging differed, we examined whether the group randomised to treatment was not as ill and therefore survived longer. The following points make this unlikely: (1) all patients had metastatic disease at recruitment and

TABLE III—SURVIVAL (MONTHS)

—	Control	Intervention
Survival from:		
Study entry to death*	18·9 (10·8)	36·6 (37·6)
Initial medical visit to death	81·2 (53·9)	94·6 (61·0)
First metastasis to death†	43·2 (20·5)	58·4 (45·4)

Mean (SD).
*$p < 0.0001$, Cox; $p < 0.005$, log-rank. †$p < 0.01$, Cox; $p < 0.04$, log-rank.



Kaplan-Meier survival plot.

A = control (n = 36), B = treatment (n = 50), and * = overlapping control and treatment probabilities of survival. Some points represent more than 1 case.

randomisation, and therefore had a fairly uniform prognosis; (2) there were no differences in other important prognostic variables; and (3) although initial staging was, as expected, significantly correlated with time from initial medical visit to date of first metastasis (Spearman's $r = -0.42$, $n = 70$, $p < 0.0003$), this variable was not correlated with any survival variables, including the outcome variable that differentiated between treatment and control groups—ie, time from entry to date of death ($r = 0.03$, $n = 70$).

To address this potential problem directly, the between-group differences were examined controlling for initial staging. The difference in survival from randomisation to death between the treatment and control groups remained highly significant ($n = 70$, $p < 0.0001$). Staging had little influence on this survival variable ($p < 0.86$). Likewise the difference between the dates of metastasis and death remained significant ($p < 0.02$). In addition, examination of Kaplan-Meier curves for treatment versus control patients matched-for-initial-staging revealed a pattern similar to that seen for the overall sample in the figure. Survival between intervention and control groups was different within each homogeneous staging group. This analysis indicated that initial staging differences do not account for the observed differences in survival between the groups.

Although there were no significant differences between treatment and control groups in chemotherapy and irradiation before randomisation, we tested the significance of the main effect for survival while controlling for each of these variables with the O'Brien procedure, entering the medical treatment variable first and then group status. In each case the treatment/control difference held. Of most interest was the significance of the treatment/control difference in survival after entering those variables that were close to being significantly different: days of irradiation ($n = 69$, $p < 0.0005$) and androgen ($n = 86$, $p < 0.0004$) and steroid treatment ($n = 86$, $p < 0.0004$). Differences in time from first metastasis to death also remained significant with this analysis. Thus these variables do not account for the enhanced survival.

There was variation in attendance among those randomised to group therapy. Illness accounted for some of this variation. Indeed, 15 patients in the treatment group and 8 controls died during the year. Some other patients moved away or were reluctant to attend their group. To examine between-group differences among those patients who where more actively involved, we did the same Cox regression analysis on the 54 patients who completed both a baseline and at least one of the three follow-up questionnaires during the year. The difference in survival time from randomisation to death between treatment and control groups again remained significant ($p < 0.0001$), even when staging was controlled ($n = 42$, $p < 0.0001$), and when log-ranks were used ($p < 0.03$).

Discussion

Patients with metastatic breast cancer randomised to weekly group therapy for a year lived significantly longer than did controls, by an average of nearly 18 months. This difference was statistically and clinically significant. Our results are consistent with but greater in magnitude than those of Grossarth-Maticek et al,[6] and overcome the problem of differences in time from initial diagnosis to study entry which limited the findings of Morgenstern et al.[7]

In agreement with Cassileth et al[16] and Jamison et al,[17] we found that a battery of extensive psychological assessments before intervention did not significantly predict survival. Indeed the only variable to affect survival time significantly was our complex psychosocial intervention. The effect of group interaction on longevity was not apparent in the year of intervention. Treatment and control groups did not diverge until about 8 months after the year was over (figure), which may be explained, as would the result of a somatic treatment, as a cumulative mild effect on time until death.

Our follow-up study was done to investigate whether psychosocial intervention, which significantly reduced anxiety, depression, and pain, would do so without having any effect on the course of the disease. We intended, in particular, to examine the often overstated claims made by those who teach cancer patients that the right mental attitude will help to conquer the disease. In these interventions patients often devote much time and energy to creating images of their immune cells defeating the cancer cells.[18] At no time did we take such an approach. The emphasis in our programme was on living as fully as possible, improving communication with family members and doctors, facing and mastering fears about death and dying, and controlling pain and other symptoms. To the extent that this intervention influenced the course of the disease, it did not do so because of any intention on the part of the therapists or the patients that their participation would affect survival time.

What could account for the differences observed? Social support may be an important factor in survival.[8,19] Even when matched for health habits, social relations affect survival.[20,21] The provision of social support for isolated individuals under stress can improve health outcome.[8] Social support is important in mediating how individuals cope with stress. For example, married cancer patients survive longer than unmarried patients.[22] In our study there was a higher proportion of married patients in the control group (70% *vs* 57%). The fact that treatment patients had longer survival may indicate the efficacy of psychosocial intervention. One role of the group might have been to provide a place to belong and to express feelings.[23] Clearly the patients in these groups felt an intense bonding with one another and a sense of acceptance through sharing a common dilemma. 1 patient with oesophageal strictures secondary to irradiation described her sense of estrangement

from the world; while struggling to swallow soup at a restaurant, she thought: "These people don't realise how fortunate they are just to be able to eat". The therapy group patients visited each other in hospital, wrote poems, and even had a meeting at the home of a dying member. Thus the groups countered the social alienation that often divides cancer patients from their well-meaning but anxious family and friends.

Involvement in the group may have allowed patients to mobilise their resources better, perhaps by complying more vigorously with medical treatment or by improving appetite and diet through reduced depression. Treated patients learnt about hypnosis for pain control and therefore may have been more able to maintain exercise and other routine activities. Neuroendocrine and immune systems may be a major link between emotional processes and cancer course.[19,24] Future studies of the impact of psychosocial interventions on medical illness might profitably examine variables such as compliance, health habits, diet, and immune and neuroendocrine function.

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REFERENCES

1. Ferlic M, Goldman A, Kennedy BJ. Group counseling in adult patients with advanced cancer. *Cancer* 1979; 43: 760.
2. Gustafson J, Whitman H. Towards a balanced social environment on the oncology service. *Soc Psychiatry* 1978; 13: 147.
3. Wood PE, Milligan J, Christ D, Liff D. Group counseling for cancer patients in a community hospital. *Psychosomatics* 1978; 19: 555.
4. Spiegel D, Bloom J. Group therapy and hypnosis reduce metastatic breast carcinoma pain. *Psychosom Med* 1983; 45: 333.
5. Forester B, Kornfeld DS, Fleiss JL. Psychotherapy during radiotherapy: effects on emotional and physical distress. *Am J Psychiatry* 1985; 142: 22.
6. Grossarth-Maticek R, Schmidt P, Veter H, Arndt S. Psychotherapy research in oncology. In: Steptoe A, Mathews A, eds. Health care and human behaviour. London: Academic Press, 1984: 325.
7. Morganstern H, Gellert GA, Walter SD, Ostfeld AM, Siegel BS. The impact of a psychosocial support program on survival with breast cancer: the importance of selection bias in program evaluation. *J Chron Dis* 1984; 37: 273.
8. Rodin J. Managing the stress of aging: the role of control and coping. In: Levine S, Ursin R, eds. Coping and Health. London: Plenum, 1980: 171.
9. House JS, Landis KR, Umberson D. Social relationships and health. *Science* 1988; 241: 540.
10. Spiegel D, Bloom J, Yalom ID. Group support for patients with metastatic breast cancer. *Arch Gen Psychiatry* 1981; 38: 527.
11. Kamby C, Rose C, Ejlertsen B, Andersen J, Birkler NE, Rytter L. Stage and pattern of metastases in patients with breast cancer. *Eur J Cancer Clin Oncol* 1987; 23: 1925.
12. Gaglia P, Bussone R, Caldarola B, Lai M, Jayme A, Caldarola L. The correlation between the spread of metastases by level in the axillary nodes and disease-free survival in breast cancer: a multifactorial analysis. *Eur J Cancer Clin Oncol* 1987; 23: 849.
13. Schwartz GF, Feig SA, Pathefsky AS. Significance and staging of nonpalpable carcinomas of the breast. *Surg Gynecol Obstet* 1988; 166: 6.
14. Spiegel D. The use of hypnosis in controlling cancer pain. *CA: Cancer J Clin* 1985; 35: 221.
15. O'Brien PC. A non-parametric test for association with censored data. *Biometrics* 1978; 34: 243.
16. Cassileth BR, Lusk EJ, Miller DS, Brown LL, Miller C. Psychosocial correlates of survival in advanced malignant disease? *N Engl J Med* 1985; 312: 1551.
17. Jamison RN, Burish TG, Walston KA. Psychogenic factors in predicting survival of breast cancer patients. *J Clin Oncol* 1987; 5: 768.
18. Achterberg J; Matthews-Simonton S, Simonton C. Psychology of the exceptional cancer patient: a description of patients who outlive predictive life expectancies. *Psychother Theory Res Practice* 1977; 14: 416.
19. Kennedy S, Kiecolt-Glaser JK, Glaser R. Immunological consequences of acute and chronic stressors: mediating role of interpersonal relationships. *Br J Med Psychol* 1988; 61: 77.

References continued at foot of next column

GRANULOCYTE COLONY-STIMULATING FACTOR AND NEUTROPHIL RECOVERY AFTER HIGH-DOSE CHEMOTHERAPY AND AUTOLOGOUS BONE MARROW TRANSPLANTATION

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Summary Granulocyte colony-stimulating factor (G-CSF) was administered by continuous subcutaneous infusion to 15 patients with non-myeloid malignancies treated by high-dose chemotherapy and autologous bone marrow infusion. G-CSF was given at variable dosage based on neutrophil count. Sustained serum levels of G-CSF were achieved. Neutrophil recovery was accelerated in G-CSF treated patients compared with 18 historical controls and exceeded $0{\cdot}5 \times 10^9$/l at a mean of 11 days after marrow infusion compared with 20 days for controls, a significant difference. This reduction led to significantly fewer days of parenteral antibiotic therapy, 11 versus 18 days in controls, and less isolation in reverse-barrier nursing, 10 versus 18 days.

Introduction

THE dose of most anticancer agents is limited by myelosuppression.[1] Drug dose is an important factor in tumour response[2] and one approach to circumvent dose limits is haemopoietic rescue by autologous bone marrow.[3-6] After such treatment there is a prolonged period of profound neutropenia[3-5] during which patients are at risk of bacterial and fungal infection.[6,7] Neutrophilic granulocyte production is stimulated by a haemopoietic growth factor, granulocyte colony-stimulating factor (G-CSF).[8] The administration of G-CSF leads to a dose-dependent rise in peripheral blood neutrophils and reduces the period of neutropenia after standard-dose cytotoxic therapy.[9-11] G-CSF 10 µg/kg per day or higher by continuous subcutaneous infusion abrogates the neutropenia seen after melphalan treatment.[12] In primates G-CSF reduces the period of neutropenia after total body irradiation and autologous bone marrow infusion.[13] Our objectives were to

D. SPIEGEL AND OTHERS: REFERENCES—*continued*

20. Berkman LF, Syme SL. Social networks, host resistance, and mortality: a nine-year follow-up study of Alameda County residents. *Am J Epidemiol* 1979; 109: 186.
21. House JS, Robbins C, Metzner HL. The association of social relationships and activities with mortality: prospective evidence from the Tecumseh community health study. *Am J Epidemiol* 1982; 116: 123.
22. Goodwin JS, Hunt WC, Key CR, Samet JM. The effect of marital status on stage, treatment and survival of cancer patients. *JAMA* 1987; 258: 3125.
23. Friedman LC, Baer PE, Nelson DV, Lane M, Smith FE, Dworkin RJ. Women with breast cancer: perception of family functioning and adjustment to illness. *Psychosom Med* 1988; 50: 529.
24. Pennebaker JW, Kiecolt-Glaser JK, Glaser R. Disclosure of traumas and immune function: health implications for psychotherapy. *J Consult Clin Psychol* 1988; 56: 239.

Correlation of Stress Factors With Sustained Depression of Natural Killer Cell Activity and Predicted Prognosis in Patients With Breast Cancer

By S. Levy, R. Herberman, M. Lippman, and T. d'Angelo

Natural killer (NK) cell activity and pyschological status were measured at baseline and at 3 months into treatment, as part of the National Cancer Institute (NCI) Protocol 79-C-111, randomizing breast cancer patients to lumpectomy/radiation v mastectomy. Patients who were found to have positive axillary lymph nodes also received combination chemotherapy (Adriamycin [Adria Laboratories, Columbus, OH], plus Cytoxan [Mead Johnson Pharmaceuticals, Evansville, IN] or methotrexate, plus 5-fluorouracil [5-FU]). Seventy-five patients were entered onto this behavioral immunology protocol at the time of data analysis. We reported in an earlier publication that NK activity was an important predictor of patient baseline prognosis relevant to nodal status. In that study, by using multiple regression analyses, 51% of the baseline NK activity variance could be accounted for by entering three distress indicators into the equation (patient "adjustment," lack of social support, and fatigue/depression symptoms). On reassessment of NK activity after 3 months, it was found that NK activity was not affected by the interim administration of chemotherapy and/or radiotherapy. However, consistent with our earlier findings, NK activity levels remained markedly lower in patients with positive nodes than in patients with negative nodes (at 60 to 1 effector to target cell [E:T] ratio, mean of 18% lytic activity v mean of 31% lytic activity [$t = 1.87$, $P < .05$]). Even though average levels of NK activity were lower for patients with more tumor burden, there was still a substantial range of NK activity levels within the node positive patient group, as well as within the patient group as a whole. We hypothesized that differences in levels of NK activity could be predicted on the basis of baseline distress factors found to be significant in our earlier report. In fact, we found that we could account for 30% of NK activity level variance at 3 months follow-up on the basis of baseline NK activity, fatigue/depression, and lack of social support. Therefore, although neither radiation nor chemotherapy appeared to affect NK activity, tumor burden was again clearly associated with NK activity levels, and a significant amount of baseline and 3-month NK activity could be predicted on the basis of CNS-mediated effects. At the least, such factors provide a psychological marker of host biological status.

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RECENTLY, links have been made within experimental animal models between helpless behavior in the face of stressful impingement, depletion of central neurotransmitter levels,[1,2] alterations in various stress hormones (including endogenous opioids),[3,4] suppression of lymphocyte function (including natural cytotoxic action by natural killer [NK] cells,[4,5] and reduced in vivo and in vitro clearance of tumor cells, including tumor cells sensitive to NK activity.[1-5] It might be expected that stressful impingement and lack of control would affect humans in a similarly negative fashion. Such events are also likely mediated by stress hormones and analogous neurochemical and immunological changes, as have been seen in the studies on murine systems cited above. However, evidence for this possibility, particularly in cancer patients, is limited.[6] There have been human studies carried out with healthy donors that have demonstrated an association between various stressors (eg, examination stress or other environmental events) and significantly reduced NK activity relevant to prestress states.[7-9] Alternatively, Kiecolt-Glaser et al[10] demonstrated the enhancement of NK activity under controlled experimental conditions in a sample of elderly subjects trained in the technique of systematic relaxation. But again, although such studies demonstrate an association, and even a causal direction, between psychological conditions and NK activity, they have not addressed the question of biological vulnerability relevant to dis-

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ease end points in cancer or other immunologically relevant disease populations.

Levy et al[11] recently reported that peripheral NK activity was an important predictor of patient prognostic risk at baseline relevant to axillary nodal status. Specifically, NK activity was significantly associated with axillary nodal status in a sample of 75 primary breast cancer patients (stages I and II) being treated at the National Cancer Institute (NCI), National Institutes of Health (NIH). Lower levels of NK lytic activity at the time of primary treatment were associated with greater numbers of axillary lymph nodes positive for cancer. By using standard multiple regression analysis,[12] these investigators also found that they could account for 51% of the NK activity variance on the basis of three "distress" factors. Patients who were rated as "adjusted" by others, ie, who appeared to an observer as undistressed, patients who complained about a lack of social support in their family environments, and patients who responded with a listless, apathetic response pattern, tended to have lower levels of NK activity. It should be emphasized—as demonstrated by Derogatis et al[13] and Visintainer and Casey[14]—that adjustment does not appear to be clinically adaptive at this stage of the disease process. Such a response pattern appears to be more akin to passive stoicism than a more active adaptation strategy.

It should also be pointed out that we selected these three psychological factors to enter into the prediction equation based on the significance of simple correlations with NK activity or on the basis of theoretical and clinical significance. Thus, fatigue ($r = -.33$, $P < .05$) and the global adjustment score ($r = -.68$, $P < .001$) were entered based on statistical significance. The social support variable, while not statistically associated with NK activity in any simple or direct way, was also entered into the predictor model because of recent evidence in the literature[15-19] that this factor has survival value. The social support variable was correlated with nodal status ($r = .23$, $P < .01$). Since higher scores on this measure indicate less perceived support within the family, the association with nodal status is in the expected direction. None of the other psychosocial variables were correlated with nodal status. As discussed in our previous work,[11] it appeared that NK activity was more directly associated with the psychological factors, and NK activity, itself, was strongly associated with nodal status. There appeared to be little direct association between psychological factors and stage of disease, apart from NK activity as potential mediator of host status.

Because we wanted to examine the short-term effects of biological treatments (radiotherapy and/or chemotherapy) on natural immunity, patients were followed over a 3-month period, and NK activity was again assessed at the end of the 3 months. The predictive power of baseline psychological and behavioral measures in accounting for NK activity variance on follow-up is reported here. We hypothesized that variation in NK activity could be predicted on the basis of baseline distress factors that had been significant in our earlier report. The ultimate aim of this ongoing work is to test whether such factors independently account for a significant amount of outcome variance in breast cancer patients, as reflected by time to disease recurrence. This issue will require longer follow-up, and will be addressed in future analyses.

MATERIALS AND METHODS

Patients

Seventy-five women with recently diagnosed stage I or stage II breast cancer, admitted to the NIH Clinical Center and participating in a randomized trial, were entered onto this behavioral immunology protocol. This study was part of an ongoing clinical trial (NCI Protocol 79-C-111) comparing the effectiveness of modified radical mastectomy v wide excision plus radical radiotherapy, including axillary lymph node dissection for both groups of patients. The mean age of these patients was 52, with a range of 28 to 74 years of age.

At the time of primary treatment, all patients underwent surgery, either axillary nodal dissection plus mastectomy, or lumpectomy and nodal dissection. All baseline interview and testing data were obtained just before patients were discharged from the hospital. Thus, all patients had had surgery approximately five to seven days before the interview (mastectomy plus axillary dissection or nodal dissection alone). No patients had yet received chemotherapy or radiation treatment. We selected this time point for baseline measurement because sufficient time had elapsed after surgery to allow for immune recovery,[20] and immunological status was not compromised in any patient by further chemical or radiological treatment. Axillary nodes were then staged by pathological exam, and within one to two days postinterview, information on the number of nodes positive was obtained from the patients' medical charts. Thus, all interviews were conducted without the interviewer or patient knowing the patient's nodal status. Patients who were found to have positive axillary lymph nodes subsequently received combination chemotherapy: Adriamycin (Adria Laboratories, Columbus, OH),

plus Cytoxan (Mead Johnson Pharmaceuticals, Evansville, IN) or methotrexate, plus 5-fluorouracil (5-FU).

Patients were followed up and assessed at their 3-month outpatient visits in order to measure in a longitudinal fashion the effects of radiation and/or chemotherapy on peripheral NK cell activity levels, and to see if we could account for change in NK activity on the basis of baseline distress measures.

Procedures

An audiotaped, structured, and quantifiable interview concerned with the patients' delay in seeking diagnoses of initial tumor symptoms, patients' attitudes toward the disease and its treatment, as well as the patients' perceptions of interpersonal support in their environments, was administered orally by project staff. This interview schedule was used in the earlier study by Derogatis et al[13] of survival time in breast cancer patients. Section II of this interview was concerned specifically with the patient's perception of interpersonal support in her environment. Content of the eight items in this section ranged from the patient being asked to rate the quality of family relationships and to estimate the availability of help provided by family members, to the patient being asked to assess the quality of communication between herself and her family members. Each question in the interview was scored from 0 (very good) to 3 (markedly inadequate). Therefore, total scores for section II had a possible range from 0 to 24.*

In addition to this taped interview, each patient filled out a self-report form, the Profile of Mood States (POMS).[21] This self-report instrument has been used in past studies with chronically ill populations, and has both acceptable validity and reliability for measuring various mood and psychiatric states.[22,23] In addition to the structured interview and the patients' self-report, the interviewer and a second independent observer rated the patients' overall adjustment to their illness by using the Global Adjustment to Illness Scale (GAIS),[24] an observer rating scale with possible scores in deciles, ranging from 0 to 100. Each decile has a written descriptor in order to enhance the reliability of rating. Scores in the lowest decile represent a high degree of psychological disturbance; scores in the upper deciles reflect adjustment and lack of psychological symptoms in the judgment of the observer. The GAIS has been found to be a highly reliable and valid clinical rating instrument for use with chronically ill populations, including cancer patients.[24]

Measurement of NK Activity

Within 24 hours after the inpatient interview, or on the day of outpatient visit, 25 mL of heparinized blood was drawn from patients. Mononuclear cells were separated on a Ficoll-hypaque gradient and resuspended at 3×10^6 cells/mL in RPMI 1640 medium with 10% fetal calf serum, and tested in a standard four-hour ^{51}Chromium-release NK cytotoxicity assay.[25] The K562 human myeloid leukemia cell line was used as target, and target cells (2×10^6) in 0.5 mL RPMI-fetal bovine serum (FBS) were incubated with 100 to 150 mL of ^{51}Cr (sodium chromate from New England Nuclear (Boston) [Cat No. NE7-0302S], 200 to 900 mCi/mg, using 100 μCi/2 $\times$ 10^6 target cells) for 60 minutes at 37°C.

*Structured interview available from the senior author on request.

The cells were washed twice and resuspended in RPMI-FBS at a concentration of 5×10^4 cells/mL. One hundred microliters of labeled target cells were mixed with 100 μL of effector cell suspensions at various concentrations to give effector-to-target (E:T) ratios ranging from 50 to 1 to 6 to 1. The cytotoxic test was performed in microtiter plates (Titertek; Flow Labs, McLean, VA), which were centrifuged at 200 $\times$ g for 3 minutes and then incubated at 37°C in a humidified air atmosphere with 5% CO_2 for four hours. At the end of the incubation period, 100 μL of supernatant was collected from each well, using the Skatron harvesting system, and the radioactivity was counted in a gamma counter. The percentage of specific release was calculated using the following formula: 100 $\times$ [(cpm test release $-$ cmp spontaneous release)/(cpm maximal release $-$ cpm spontaneous release)], where the spontaneous and the maximal releases were obtained from labeled target cells incubated in medium alone or in a 5% Triton $\times$ 100 solution, respectively. Spontaneous release was consistently $<$ 10% of the total incorporated radioactivity, whereas the maximal release was always at least 80%.

NK activity data reported here were analyzed using percentage of cytotoxicity at the 50 to 1 E:T cell ratio.

Schedule and Dosage of Chemotherapy and Radiotherapy

Complete details of treatment schedule and dose intensity have recently been published,[36] and are available to the reader. Therefore, such details of treatment will not be further elaborated here.

RESULTS

NK Activity as a Function of Adjuvant Therapeutics

As shown in Table 1, the mean levels of NK activity in this sample of breast cancer patients did not significantly change over a 3-month period, even in groups of patients receiving adjuvant chemotherapy or radiotherapy. As previously reported for the baseline tests,[11] node-positive patients continued to have comparatively low levels of NK activity at 3-month follow-up. In fact, overall mean NK activity levels for node-positive *v* node-negative patients across the two measurement periods were $\bar{X}$ = 18% lytic activity *v* $\bar{X}$ = 31% lytic activity (t = 1.83, $P < .05$), respectively. For node-positive patients, irrespective of whether they received adjuvant chemotherapy plus radiotherapy, or just adjuvant chemotherapy alone, there was no significant additional decrement to NK activity as a function of these treatments over a 3-month period. This finding of comparatively lower levels of natural cell-mediated cytotoxicity in patients with greater tumor burden is consistent with other reports in the literature.[27,28] However, in contrast with

Table 1. Mean and SD for Baseline and 3-Month NK Activity Levels for Positive and Negative Axillary Node Patients Randomized to Lumpectomy/Radiation v Mastectomy (NCI Protocol No. 79-C-111)

	Patients Randomized to Lumpectomy + Radiation Arm				Patients Randomized to Mastectomy Arm			
	− Nodes		+ Nodes		− Nodes		+ Nodes	
	Baseline	3 Months	Baseline	3 Months	Baseline	3 Months	Baseline	3 Months
N	14	14	10	10	14	14	6	6
Mean NK activity	29.8	25.1	19.5	21.6	33.5	34.5	18.6	16.6
SD	15.1	14.4	8.8	9.5	11.1	17.9	7.2	14.4

previous reports using cross-sectional designs, our data are longitudinal in nature, and demonstrate on the average a sustained suppression in such patients over time, despite the reduction in tumor burden by surgery.

NK Activity at 3 Months as a Function of Baseline NK Activity Levels and Distress Factors

Although adjuvant therapy did not seem to account for alterations in NK activity at the 3-month test period, predicting NK activity level variance from baseline to 3 months for the group as a whole, as a function of important baseline predictor variables,[11] revealed sources of NK activity variance at time 2. Table 2 shows the results of this additional stepwise multiple regression analysis.

As can be seen in Table 2, NK activity at time 1, the POMS fatigue subscale score[21] at time 1, and the patient's report of social support, together, accounted for 30% of the NK activity variance at time 2 ($F = 3.8$, $P < .02$). Clearly, the most important predictor at time 2 was NK activity level at baseline. Statistically, by entering baseline NK activity first into the equation, we simply took into account the predictive value of baseline NK activity level, and were then able to ask whether any other variable additionally accounted for NK activity variance at time 2. A trend was seen for patients who responded with a listless response pattern ($P < .07$), and who reported little in the way of social support ($P < .1$) to have altered NK activity values at follow-up, and in the same direction as previously reported.[11] Individual total scores on the relevant psychological measures could thus be entered into the regression equation, allowing for the calculation of predicted NK activity based on these scores. Again, patients reporting depressive, fatigue-like symptoms, and who complained about lack of family support at baseline, tended to show a decrease in NK activity levels at 3-month follow-up.

A most convincing demonstration that NK activity at 3 months was correlated with distress factors identified at baseline emerged from a final regression analysis. We repeated the previously reported[11] multiple regression analysis, predicting NK activity variance at time 2, based on time 2, rather than baseline, variables. The same results emerged as a function of these factors measured simultaneously with NK activity at the 3-month assessment period. That is, by using both stepwise/forced and stepwise/forward (unforced) multiple regression techniques, we could account for 44% of the NK variance (adjusted R^2) on the basis of the GAIS observer rating ($R^2 = 33\%$) (described in detail in our previous publication,[11] Fatigue scale (cumulative $R^2 = 40\%$), and perception of family support (cumulative $R^2 = 44\%$, $P < .01$). Therefore, factors that were significant in accounting for NK activity variance at baseline assessment while the patient was still hospitalized were ap-

Table 2. Stepwise Multiple Regression Analysis Predicting Time 2 NK Activity

Variable	ß	Multiple R	R^2	Significance (P)
NK at time 1	−.60	.38	.15	.008
Fatigue (POMS subscale)	−.83	.47	.22	.07
Interpersonal perception	1.56	.53	.29	.1
Constant	15.58			

NOTE. $F = 3.8$, $P < .02$.

parently still operative, and with the same direction of effect, 3 months posthospital discharge.

DISCUSSION

We were somewhat surprised by the finding that NK activity was seemingly unaffected by interim treatments since a common effect of such therapy is the suppression of lymphocyte activity. Although Frei[29] has recently noted that intermittent treatment with chemotherapeutic agents allows for interval recovery and indeed, in some cases, promotes overshoot of immune response, the use of such agents as methotrexate is generally viewed as immunosuppressive.[30] Although given our measurement interval, we could not assess acute, short-term treatment-related suppression of NK activity, whatever effects might have occurred were clearly time-limited, allowing for interim recovery to baseline levels. In addition, it is also possible that since the radiotherapy was relatively localized, such circumscribed treatment did not have appreciable systemic immunosuppressive effects.† Assuming a role for natural immunity in tumor control, the apparent resistance of these cells to long-term radiation or chemical suppression may be a clinically significant observation.

Clinically, these findings indicate that patients' scores on these mood and social support variables could be entered into the statistical model developed and used to predict the direction of NK activity 3 months postbaseline treatment. If NK cells play a surveillance role in curbing malignant cell spread,[25] then being able to predict relatively low NK activity level is not only scientifically interesting, but may be a clinically useful finding. That is, scores on these psychological measures may in fact be viewed as markers of biological risk status. Breast cancer patients scoring in the "risk" range on measures of fatigue/depression and social support perception could be identified as biologically vulnerable. Such patients would be suitable candidates for behavioral intervention strategies. Whether such intervention would affect biological status is an unanswered question at this point.

The aggregate of recent evidence from a variety of sources supports the conclusion that there likely is a psychosocial risk profile for at least breast cancer and malignant melanoma patients consistent with our findings, generally characterized as stoic and passive.[13,14,31-34] In addition, there is ample evidence from large, prospective community studies[15-19] that lack of social support is associated with increased morbidity and mortality, including cancer incidence and death from cancer as end points, in various populations.

This series of findings from ongoing NCI work, reported here and elsewhere,[7] suggests one mediating pathway linking these behavioral and social factors with host risk. Recent laboratory investigations[36-39] have demonstrated that tumor-relevant lymphocyte subpopulations, including NK cells, have receptors for various neuropeptides, including endogenous opioids. Recent animal research[1-5,39] has shown that a passive behavioral response pattern when the organism is confronted with a stressor is a potent elicitor of β-endorphin, as well as other "stress" hormones. Although the notion that passive patients fare worse has been part of our clinical lore for decades, it has only been in recent years that the behavioral, immunological, and endocrinological technology is available to begin to trace mediating paths linking higher cortical function and the host's behavior with cancer prognosis and survival in clinical populations. The findings reported here may supply one answer to this complex puzzle.

†It should be noted that all tests for NK-mediated cytotoxicity were carried out in vitro. While all cells were brought up to a standard concentration, there was still a wide range of lytic activity across patients. At least on a per cell basis, radiotherapy and chemotherapy, per se, did not seem to affect NK cell functional potency.

REFERENCES

1. Sklar L, Anisman H: Stress and coping factors influence tumor growth. Science 205:513-515, 1979

2. Weiss J, Glazer H, Pohorecky L, et al: Effects of chronic exposure to stressors on avoidance-escape behavior and on brain norepinephrine. Psychosom Med 37:522-534, 1975

3. Shavit Y, Lewis J, Terman G, et al: Opioid peptides mediate the suppressive effect of stress in natural killer cell cytotoxicity. Science 223:188-190, 1984

4. Greenberg A, Dyck D, Sandler L: Opponent processes, neurohormones and natural resistance, in Fox B, Newberry B (eds): Impact of Psychoendocrine Systems in Cancer and Immunity. Toronto, Hogrefe, 1984, pp 255-258

5. Laudenslager M, Ryan S, Drugan R, et al: Coping and immunosuppression: Inescapable but not escapable shock suppresses lymphocyte proliferation. Science 221:568-570, 1983

6. Levy S: Behavior and Cancer. San Francisco, Jossey-Bass, 1985

7. Kiecolt J, Glaser R, Strain E, et al: Modulation of cellular immunity in medical students. J Behav Med 9:5-21, 1986

8. Kiecolt-Glaser J, Ricker D, George J, et al: Urinary cortisol levels, cellular immunocompetency, and loneliness in psychiatric inpatients. Psychosom Med 46:15-23, 1984

9. Levy S, Herberman R, Maluish A, et al: Stress, coping, and illness in relation to persistently low natural killer cell activity. Presented at Meeting of the American Psychological Association, Washington, DC, 1986

10. Kiecolt-Glaser J, Glaser R, Williger D, et al: Psychosocial enhancement of immunocompetence in a geriatric population. Health Psychol 4:25-41, 1985

11. Levy S, Heberman R, Maluish A, et al: Prognostic risk assessment in primary breast cancer by behavioral and immunological parameters. Health Psych 4:99-113, 1985

12. Cohen J, Cohen P: Applied Multiple Regression/Correlation Analysis for the Behavioral Sciences. New York, Wiley, 1975

13. Derogatis L, Abeloff M, Melisaratos N: Psychological coping mechanisms and survival time in metastatic breast cancer. JAMA 242:1504-1508, 1979

14. Visintainer MA, Casey R: Adjustment and outcome in melanoma patients. Presented at meeting of the American Psychological Association, Toronto, 1984

15. Berkman L, Syme L: Social network, host resistance, and mortality: A nine-year follow-up study of Alameda County residents. Am J Epidemiol 2:186-204, 1979

16. Welin L, Svärdsudd K, Ander-Peciva S, et al: Prospective study of social influences on mortality: The study of men born in 1913 and 1923. Lancet 34:915-918, 1985

17. House J, Robbins C, Metzner H: The association of social relationships and activities with mortality: Prospective evidence from the Tecumseh community health study. Am J Epidemiol 116:123-140, 1982

18. Blazer D: Social support and mortality in an elderly community population. Am J Epidemiol 115:684-694, 1982

19. Cassel J: The contribution of the social environment to host resistance. (The fourth Wade Hampton Frost lecture.) Am J Epidemiol 104:107-123, 1976

20. Tonnesen E, Mickley H, Grunnet N: Natural killer cell activity during premedication, anaesthesia, and surgery. Acta Anaesth Scand 27:238-241, 1983

21. McNair D, Lorr M, Droppleman L: EITS manual for the profile of mood states. San Diego, Educational Testing Services, 1971

22. Goldberg L: Objective diagnostic tests and measures, in Rosenzweig M, Porter L (eds): Annual Review of Psychology. San Francisco, Annual Reviews, 1974, pp 343-366

23. Covi L, Lipman R, Frighi L, et al: Transcultural comparability of outpatient self-ratings: II. The profile of mood states (POMS). Drugs Exp Chem R 1:141-151, 1977

24. Morrow G, Feldstein M, Adler L, et al: Development of brief measures of psychosocial adjustment in medical illness applied to cancer patients. Gen Hosp Psychiatry 3:79-88, 1981

25. Ortaldo J, Bonnard G, Herberman R: Cytotoxic reactivity of human lymphocytes cultured in vitro. J Immunol 19:1351-1357, 1977

26. Lippman M, Edwards B, Findlay P, et al: Influence of definitive radiation therapy for primary breast cancer on ability to deliver adjuvant chemotherapy. NCI Monographs 1:99-104, 1986

27. Takasugi M, Ramseyer A, Takasugi J: Decline of natural nonselective cell-mediated cytotoxocity in patients with tumor progression. Cancer Res 37:413-418, 1977

28. Steinhauer E, Doyle A, Reed J, et al: Defective natural cytotoxicity in patients with cancer: Normal number of effector cells but decreased recycling capacity in patients with advanced disease. J Immunol 129:2255-2259, 1982

29. Frei E: Chemoimmunotherapy: Strategy considerations, in Reif A, Mitchell M (eds): Immunity to Cancer. Orlando, FL, Academic, 1985, pp 635-642

30. Pratt W, Ruddon R: The Anti-Cancer Drugs. New York, Oxford University, 1979

31. Jensen M: Psychobiological factors in the prognosis and treatment of neoplastic disorders. Doctoral dissertation, Department of Psychology, Yale University, 1984

32. Rogentine G, Van Kammen D, Fox B, et al: Psychological factors in the prognosis of malignant melanoma: A prospective study. Psychosom Med 41:647-655, 1979

33. Pettingale K, Morris T, Greer S, et al: Mental attitudes to cancer: An additional prognosis factor. Lancet 750, 1985

34. Greer S, Morris T, Pettingale K: Psychological response to breast cancer: Effects on outcome. Lancet 2:785-787, 1979

35. Faith R, Liang H, Murgo A, et al: Neuroimmunomodulation with enkephalins: Enhancement of human natural killer (NK) cell activity in vitro. Clin Immunol Immunopathol 31:412-418, 1984

36. Weber R, Pert C: Opiatergic modulation of the immune system, in Müller E, Genazzani A (eds): Central and Peripheral Endorphins: Basic and Clinical Aspects. New York, Raven, 1984, pp 35-42

37. Wybran J, Appelboom T, Famaey J, et al: Suggestive evidence for receptors for morphine and methionine-enkephalin or normal human blood T lymphocytes. J Immunol 123:1068-1070, 1979

38. Brown SL, Van Epps D: Suppression of T lymphocyte chemotactic factor production by the opioid peptides B-endorphin and MET-enkephalin. J Immunol 134:3384-3390, 1985

39. Miczek L, Thompson M, Schuster L: Opioid-like analgesia in defeated mice. Science 215:1520-1522, 1982

REPORTS

Stress and Immune Responses After Surgical Treatment for Regional Breast Cancer

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Background: Adults who undergo chronic stress, such as the diagnosis and surgical treatment of breast cancer, often experience adjustment difficulties and important biologic effects. This stress can affect the immune system, possibly reducing the ability of individuals with cancer to resist disease progression and metastatic spread. We examined whether stress influences cellular immune responses in patients following breast cancer diagnosis and surgery. *Methods:* We studied 116 patients recently treated surgically for invasive breast cancer. Before beginning their adjuvant therapy, all subjects completed a validated questionnaire assessing the stress of being cancer patients. A 60-mL blood sample taken from each patient was subjected to a panel of natural killer (NK) cell and T-lymphocyte assays. We then developed multiple regression models to test the contribution of psychologic stress in predicting immune function. All regression equations controlled for variables that might exert short- or long-term effects on these responses, and we also ruled out other potentially confounding variables. *Results:* We found, reproducibly between and within assays, the following: 1) Stress level significantly predicted lower NK cell lysis, 2) stress level significantly predicted diminished response of NK cells to recombinant interferon gamma, and 3) stress level significantly predicted decreased proliferative response of peripheral blood lymphocytes to plant lectins and to a monoclonal antibody directed against the T-cell receptor. *Conclusions:* The data show that the physiologic effects of stress inhibit cellular immune responses that are relevant to cancer prognosis, including NK cell toxicity and T-cell responses. Additional, longitudinal studies are needed to determine the duration of these effects, their health consequences, and their biologic and/or behavioral mechanisms. [J Natl Cancer Inst 1998; 90:30–6]

A diagnosis of cancer and cancer treatments are objective, negative events in an individual's life. Although negative events do not always produce stress and a lowered quality of life, data from many studies document severe, acute stress at cancer diagnosis *(1)* and during recovery *(2)*. The negative psychologic responses of individuals with cancer to the diagnosis and treatment are important in their own right because these responses are targets for cancer control efforts *(3,4)*. In addition, data suggest that stress responses are accompanied by nonrandom (i.e., correlated) negative changes in a broad range of immune responses. This study examines from a biobehavioral perspective whether stress influences cellular immunity in women with breast cancer after diagnosis of breast cancer and during the postsurgical period *(5)*.

Meta-analyses *(6,7)* suggest that psychologic stress and the experience of life stressors are reliably associated with negative immune alterations in noncancer subjects; i.e., "higher" levels of stress (e.g., self-reports of stress or negative affects, such as sadness or clinical diagnoses of depression) are related quantitatively and functionally to "reduced" cellular immune responses, such as lowered natural killer (NK) cell lysis. This effect has been found regularly for individuals in the midst of chronic stressors, and some of the largest responses and changes have been found for lengthy stressors and those that have interpersonal components.

Illustrative data come from Kiecolt-Glaser, Glaser, and colleagues *(8–11)*, who have followed individuals during the long, stressful experience of giving care to a spouse diagnosed with Alzheimer's disease. Not surprisingly, caregivers report high levels of distress and negative affect as they cope with their relative's difficult behavior and mental deterioration *(8)*. Moreover, these researchers have found, for example, that NK cells obtained from caregivers are less responsive to the cytokine recombinant interferon gamma (rIFN γ) and recombinant interleukin 2 (rIL-2) than are cells obtained from matched community control subjects *(9)*. In addition, these highly stressed subjects have a poorer proliferative response to mitogens *(8)*, exhibit substantial deficits in the antibody and virus-specific T-cell responses to an influenza virus vaccine *(10)*, and demonstrate stress-related defects in wound repair *(11)*.

There are fewer data on the relationship between stress and immunity among cancer patients. Levy et al. *(12)* reported on these relationships in 66 women with stage I or II breast cancer 3 months after treatment (lumpectomy or mastectomy with or without adjuvant therapy). In ad-

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See "Notes" following "References."

dition to finding that estrogen receptor status predicted NK cell lysis, these researchers found that social support—a variable hypothesized to *reduce* stress—contributed significantly to a regression model predicting *higher* NK cell activity. These findings suggest that how a person responds to stress may also influence how stress, in turn, influences the immune response.

There is considerable evidence that patients with cancer express abnormal cellular immune responses; these abnormal responses have been found in patients with many different types of cancer *(13–15)*, including breast cancer *(16,17)*. Stressors are not generic, and they would not be expected to have identical physiologic outcomes. So too, the immune response involves a cascade of responses and events that can occur over time. For these reasons, we used a homogeneous breast cancer subject sample and timing of assessment to test the relationship between stress and several components of the cellular immune response, including NK cell and T-cell functions.

Women who had been diagnosed with breast cancer and who had undergone surgery for the breast cancer were studied before they began adjuvant therapy. Since we were interested in the contribution of stress in predicting an immune response above and beyond known correlates, we controlled for naturally occurring factors in our statistical analyses that affect the immune responses—specifically, age, disease stage (lymph node status), and recovery (days since surgery) *(18)*. Because the immune system contains a considerable amount of redundancy, we focused on three components that would each provide important, but complementary, information.

First, we measured NK cell lysis. We chose to measure NK cell lysis because those cells are believed to act early in the immune response and they have been demonstrated to play an important role in immune surveillance against tumors and virally infected cells *(19–21)*. Second, we measured the ability of the NK cells to respond to rIFN γ and rIL-2. It has been shown that lymphokine-activated killer (LAK) cells are highly cytotoxic against a wider variety of tumor cells than those lysed by resting NK cells *(22)*, an effect also observed in patients with breast cancer *(23)*. Finally, to obtain information on the T-cell response, we measured the response of peripheral blood leukocytes (PBLs) to two mitogens—phytohemagglutinin (PHA) and concanavalin A (Con A)—and we induced proliferation by stimulating the T cells with a monoclonal antibody (MAb) to the T-cell receptor.

Subjects and Methods

Patient Eligibility and Data Collection

Participants were 116 women who had been diagnosed with invasive breast cancer and who were surgically treated within the last 4 months but who had not yet begun adjuvant treatment. Women were from 14 to 101 days (mean = 37 days; median = 33 days) after surgery for stage II (70%) or III (30%) invasive breast cancer. We used the American Joint Committee on Cancer and the International Union Against Cancer staging system. The women ranged in age from 31 to 84 years (mean = 52 years). Recruited consecutively from mid-1994 to early 1997, the majority (82%) were being treated at a National Cancer Institute-designated, university-affiliated Comprehensive Cancer Center, and the remainder (18%) were receiving treatment at local community hospitals. All women came to the General Clinical Research Center at the university where psychologic, behavioral, and medical data were collected and a 60-mL blood sample was taken from them. Assessments were conducted between 8:00 AM and 12:00 AM to reduce diurnal variability.

Stress Measure

The Impact of Event Scale (IES) *(24)* is a standardized self-report questionnaire used to examine intrusive thoughts ("I had dreams about being a cancer patient." "Other things kept making me think about cancer") and avoidant thoughts and actions ("I tried not to talk about it." "I was aware that I still had a lot of feelings about cancer, but I didn't deal with them") concerning cancer. Fifteen items are used, and women rate each event or feeling in terms of the frequency of occurrence (i.e., "not at all," "rarely," "sometimes," and "often") during the previous 7 days. Scores range from 0 to 75. For this sample, descriptive statistics were as follows: range, 0–65; mean = 26; median = 25; and standard deviation = 15.2. The scale has satisfactory reliability with internal consistency of .78–.82 and a 2-week test–retest reliability of .79–.89, respectively. The validity of the measure is suggested by data indicating that individuals who experience involuntary, distress-related thoughts following traumatic life events are also those who suffer the greatest negative effects psychologically [e.g., (2)].

Immune Assays

Blood cell separation. PBLs were isolated from 60 mL of venous blood by use of Ficoll gradients (Pharmacia Biotech, Inc., Piscataway, NJ). The isolated leukocytes were then washed in calcium- and magnesium-free phosphate-buffered saline and counted on a Coulter counter (Coulter Corp., Miami, FL). Aliquots of 8×10^6 isolated PBLs were suspended again in 0.8 mL of RPMI-1640 medium supplemented with 10% fetal bovine serum, 0.75% sodium bicarbonate, 2 m*M* L-glutamine, and 10 μg/mL of ciprofloxacin.

Quantification of total T lymphocytes, T-cell subsets, and NK cells. Isolated PBLs were absorbed with MAbs conjugated to either fluorescein isothiocyanate or rhodamine according to the cell surface marker being studied: total T cells (CD3, fluorescein isothiocyanate), T4 subset (CD4, rhodamine), T8 subset (CD8, fluorescein isothiocyanate), and NK cells (CD56, rhodamine). All MAbs were purchased from Coulter Corp. Briefly, 0.5×10^6 cells were incubated with the MAb for 15 minutes at room temperature. After the incubation, the cells were fixed, and the red blood cells were lysed with Optilyse C, a buffered solution containing 1.5% formaldehyde, according to the manufacturer's instructions (Coulter Corp.). Samples were analyzed with the use of a Coulter EPICS Profile II flow cytometer as described previously *(8)*.

NK cell cytotoxicity. To determine NK cell activity, a microtiter ^{51}Cr-release cytotoxicity assay was used as described previously *(9,25)*. The target cells used were K-562 cells, an NK cell-sensitive human myeloid cell line. Target cells, labeled overnight for 16 hours with ^{51}Cr, were placed in triplicate wells of 96-well V-bottom plates, and PBLs were added, resulting in effector-to-target (E:T) cell ratios of 100:1, 50:1, 25:1, 12.5:1, and 6.25:1.

NK cell response to cytokines. Procedures for treatment of PBLs with rIFN γ and rIL-2 involved preparing isolated PBLs at a concentration of 3×10^6 cells/mL in complete RPMI-1640 medium and then seeding the cells into three replicate tissue culture tubes (Falcon, Becton Dickinson and Co., Lincoln Park, NJ) at 6×10^6 cells per tube. Cells were incubated in complete RPMI-1640 medium alone or complete medium supplemented with 250 IU/mL rINF γ or 60 IU/mL rIL-2 (Genzyme, Boston, MA). Cell suspensions were gently mixed and then incubated at 37 °C in an atmosphere of 5% CO_2 for 65 hours. For the assay, triplicate aliquots of cell suspensions were placed in wells of V-bottom plates, with E:T cell ratios of 50:1, 25:1, 12.5:1, 6.25:1, or 3.13:1. In addition, six wells with target cells and medium only and target cells with detergent (5% sodium dodecyl sulfate in phosphate-buffered saline) were prepared to determine spontaneously released chromium and maximal lysis, respectively. The plates were centrifuged at 300*g* for 5 minutes at 20 °C to bring the effector and target cells into close contact; they were then incubated at 37 °C in an atmosphere of 5% CO_2 for 5 hours. After this incubation, the plates were centrifuged at 300*g* for 5 minutes at 20 °C. 100 μL of supernatant was collected from each well, and counts per minute were determined by use of a Beckman 9000 gamma counter (Beckman Instruments, Inc., Fullerton, CA) as described previously *(9,26)*.

Blastogenic response to PHA, Con A, and MAb to the T3 receptor. The concentrations for PHA and Con A used were 2.5, 5.0, and 10.0 μg/mL. To measure the blastogenic response to the MAb to the T-cell receptor, we used the following three dilutions of the purified MAb: 32:1, 64:1, and 128:1. For all three assays isolated, PBLs seeded in triplicate at 0.5×10^5 per well were incubated for 68 hours at 37 °C in 96-well flat-bottomed plates and then labeled for 4 hours with MTS, i.e., 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt

Promega Corp., Madison, WI) to measure proliferative response. Briefly, the MTS procedure is a nonradioactive calorimetric procedure that labels metabolically active cells via reduction of a colored substrate. The amount of proliferation was determined by optical density of the suspension in the well. Optical density determinations were performed by use of a Titertek Multiscan MCC microplate reader (Flow Laboratories, Inc., Finland) at a determination wavelength of 492 nm and a reference wavelength of 690 nm as has been noted *(27,28)*.

Statistical Analyses

Preliminary analyses. Before conducting the principal analyses, we checked the data for the contribution of "nuisance" variables (covariates) that could potentially be related to psychologic stress, immune outcomes, or both [*see (25)* for a discussion]. The variables examined were measures of aspirin, alcohol, caffeine, and nicotine intake; amount of sleep; plasma albumin level (as an indicator of nutritional status); incidence of recent infectious illness; and the Karnofsky performance status rating. We examined the relationships between these variables and each of the three sets of outcome variables: NK cell lysis, ability of NK cells to respond to rIFN γ and rIL-2, and the blastogenic response of PBLs to Con A, PHA, and the T3 MAb. Analysis of variance was used for the categorical independent variables, and simple correlations were used for numerically scaled independent variables.

Screening of these potential covariates involved examination of the relationships between 11 covariates and 20 dependent variables, or a total of 220 bivariate associations. Of these 220 associations, 15 were found to be statistically significant at .05 significance level. This number of significant effects is only slightly more than would be expected by chance alone (i.e., 220 × .05 = 11). Inspection of the significant relationships showed that many of them were attributable to the influence of a few outliers in the data. To be conservative, all of the regression analyses described below were run twice, once including and once excluding those covariates that had significant bivariate associations with the relevant dependent variables. In no case were results of the regression analyses significantly altered by the inclusion of the covariates. Given this fact and the consistently weak relationships of the covariates to the dependent variables, we do not report further results involving the covariates.

Principal analyses. The principal analyses assess the relationship between the IES measure of psychologic stress and the following three sets of outcome measures: 1) NK cell lysis at five E:T ratios, 2) response of NK cells to rIFN γ and rIL-2 stimulation at five E:T ratios each, and 3) the PBL blastogenic response to PHA and Con A and proliferative response to the T3 MAb at three concentrations or dilutions each.

We were interested in the role of stress in predicting these outcomes, over and above the impact of disease and recovery variables on the immune response. Thus, we chose to control for three variables: 1) age, which is associated with downregulation of the immune system; 2) disease stage, which is an indicator of the extent or burden of disease; and 3) days since surgery, which is an indicator of the degree of recovery from surgical stress and related factors (e.g., anesthesia).

Using hierarchical multiple regression (29), we tested the predictive value of psychologic stress for the measured immune outcomes. This procedure enters variables in a specified sequence and, at the final step, provides a test of the variance of the dependent variable (immune outcome) due to the predictor (stress), above and beyond the contribution of the control variables (age, stage, and days since surgery). In these regression analyses, age, days since surgery, and IES were considered as numerical variables. Stage was a categorical variable with two levels: II versus III.

For all of the analyses described below, any missing data were managed by the pairwise deletion technique, wherein each bivariate association is estimated with the use of all subjects for whom measures on both variables are available. This approach allows for more complete usage of available data than do alternative procedures (e.g., listwise deletion). For all of the dependent variables except the response of NK cells to rIFN γ, the quantity of missing data was small—with never more than 10 observations missing for any bivariate association. Effective sample sizes for the regression analyses ranged from 113 for the NK cell lysis ratios to 103 for T3 MAb values. For rIFN γ measures, sample sizes varied from 85 to 49 across the range of concentrations employed.

For each analysis, we provided three regression models: models A, B, and C. Model A includes only the control (independent) variables (i.e., age, stage, and days since surgery) in predicting the immune outcome (e.g., NK cell lysis). Predictors in model A were introduced simultaneously because we had no basis for or a strong interest in investigating their effects in any particular sequence. Model B includes the three control variables as well as the psychologic stress variable (IES) in the prediction of the immune outcome. Of particular interest in this analysis was the increment in the squared multiple correlation (R^2) from model A to model B (i.e., R^2_{B-A}), indicating variance in a dependent variable (e.g., NK cell lysis) attributable to stress (IES) beyond that explained by the control predictors. In addition, the standardized regression beta (β) for the psychologic stress variable (IES) *in model B* (i.e., β_{Stress}) indicates the magnitude and direction of the influence of this predictor on the dependent variable. The significance of the β weight was also tested. Finally, model C indicates the contribution of psychologic stress as the lone predictor; this third model provides the simple association between psychologic stress and immune function.

Results

Analyses Predicting NK Cell Lysis

Table 1 provides the results from the three models, A, B, and C, predicting NK cell lysis. For model A, in which age, stage, and days since surgery are the independent variables, R^2_A was small and nonsignificant for every E:T ratio (all F ratios were <1.0). Because the percentage of NK cells available would influence the

Table 1. Results of regression analyses for predicting natural killer (NK) cell lysis across six effector-to-target cell (E:T) ratios

	Dependent variable: NK cell lysis at E:T ratios					
	100:1	50:1	25:1	12.5:1	6.25:1	3.125:1
Model A, R^2_A*	.005	.007	.012	.015	.020	.023
Model AA, R^2_{AA}†	.085	.148	.185	.233	.250	.241
Model B‡						
R^2_B	.135	.212	.238	.268	.275	.253
R^2_{B-AA}§	.050	.064	.053	.035	.025	.012
β_{Stress}‖	−.234	−.265	−.240	−.194	−.165	−.115
$t(df = 110)$¶	−2.462	−2.921	−2.672	−2.223	−1.892	−1.280
P	.016	.004	.008	.028	.062	.204
Model C#						
R^2_C	.067	.091	.084	.066	.056	.032
$t(df = 110)$¶	−2.826	−3.338	−3.199	−2.811	−2.558	−1.867
P	.006	.002	.002	.006	.012	.066

*Model A includes the control predictors of age, stage, and days since surgery for the immune outcome, NK cell lysis. The R^2_A is the total variance in NK cell lysis explained by these three predictors.

†Model AA includes model A variables plus the control predictor percentage of NK cells for the immune outcome, NK cell lysis. The R^2_{AA} is the total variance in NK cell lysis explained by these four predictors.

‡Model B includes model AA control variables plus the stress predictor (i.e., Impact of Event Scale [IES] score) for the immune outcome, NK cell lysis. The R^2_B is the total variance in NK cell lysis explained by the four control predictors and the stress predictor.

§R^2_{B-AA} is the increment in variance due to stress only (i.e., variance beyond that explained by the control predictors) in predicting the NK cell lysis outcome.

‖β_{Stress} is the standardized regression beta (β) for the stress variable in model B. It indicates the magnitude and direction of the influence (negative) of stress on the immune outcome.

¶*df* refers to the degrees of freedom in model B.

#Model C includes stress as the only predictor of the immune outcome, NK cell lysis. The R^2_C is the total variance in NK cell lysis explained by stress; this model provides the simple association between psychologic stress and immune function.

NK cell activity as measured by lysis, we next added the percentage of NK cells, as determined by flow cytometry, into the analyses as an additional, independent control variable as shown (model AA). Across all E:T ratios, the R^2_{AA} values suggested that this variable added significant variance, as predicted, yielding R^2_{A-A} values ranging from .085 to .250.

More important was the addition of the stress variable (IES) as a predictor, shown in model B. The value of R^2_B for lysis was noticeably larger than that of R^2_{AA}, and it provided a significant increment in prediction across the E:T ratios. These data indicate that the measure of psychologic stress that was used accounted for significant variance in NK cell lysis above and beyond that explained by age, stage, days since surgery, and percentage of NK cells. Moreover, the sign of the β regression coefficient for IES was negative, as predicted, indicating that an increase in measured stress was associated with a decline in NK cell lysis. The *t* tests for these coefficients were significant at five of the six E:T ratios. Also, no other predictor in model B had a significant regression coefficient.

We also provide the regression results when only IES was used as a predictor, eliminating the control predictors from the model (model C in Table 1). These results showed that the simple association between IES and NK cell lysis was statistically significant at five of the six E:T ratios.

Table 2. Results of regression analyses for predicting natural killer (NK) cell response to recombinant interferon gamma (rIFN γ) across five effector-to-target cell (E:T) ratios

	Dependent variable: NK cell response to rIFN γ at E:T ratios				
	50:1	25:1	12.5:1	6.25:1	3.125:1
Model A, R^2_A*	.025	.097	.080	.138	.124
Model B†					
R^2_B	.041	.151	.197	.257	.208
R^2_{B-A}‡	.016	.054	.117	.119	.084
β_{Stress}§	−.128	−.244	−.358	−.358	−.301
t	−1.104	−2.190	−3.203	−3.084	−2.083
df‖	82	81	74	65	46
P	.274	.032	.002	.004	.044
Model C¶					
R^2_C	.015	.077	.149	.149	.088
t	−1.128	−2.586	−3.581	−3.343	−2.080
df‖	82	81	74	65	46
P	.264	.012	.002	.002	.044

*Model A includes the control predictors of age, stage, and days since surgery for the immune outcome, NK cell response. The R^2_A is the total variance in NK cell response explained by these three predictors.

†Model B includes model A control variables plus the stress predictor (i.e., Impact of Event Scale [IES] score) for the immune outcome, NK cell response. The R^2_B is the total variance in NK cell response explained by the three control predictors and the stress predictor.

‡R^2_{B-A} is the increment in variance due to stress only (i.e., variance beyond that explained by the control predictors) in predicting the NK cell response.

§β_{Stress} is the standardized regression beta (β) for the stress variable in model B. It indicates the magnitude and direction of the influence (negative) of stress on the immune outcome.

‖*df* refers to the degrees of freedom in model B.

¶Model C includes stress as the only predictor of the immune outcome, NK cell response. The R^2_C is the total variance in NK cell response explained by stress; this model provides the simple association between psychologic stress and immune function.

Analyses Predicting Response of NK Cells to Cytokines

Results for the NK cell response to rIFN γ are provided in Table 2 and show a similar pattern. For model A, which used age, stage, and days since surgery as the independent variables, the value of R^2_A was small to moderate, ranging from .025 to .138. When stress (IES) was added to the model B regression, the R^2 values were statistically significant at all but one E:T ratio (50:1). Furthermore, the increments in the prediction due to IES, R^2_{B-A}, were significant and ranged from .054 to .119. This value reflects the proportion of variance in the cell response accounted for by stress (IES) beyond that explained by the control variables. Again, the negative weight of β for IES in model B indicated a negative influence of psychologic stress on the response of the NK cells to rIFN γ. Again, no other predictor in model B had a significant regression coefficient. Finally, the results for model C in Table 2 showed a simple association between IES and the rIFN γ response. These correlations were significant at four of the five E:T ratios; the proportions of variance accounted for were in the range of .077 to .149.

We attempted to calculate a parallel set of regressions for the response of NK cells to rIL-2. However, cells from a large proportion of the patients (62%) had no response to rIL-2. When the regressions were conducted on data obtained from the remaining patients (38%), the addition of stress (IES) in model B produced a significant R^2 value at the 25:1 E:T ratio only. It appeared that the majority of the subjects' NK cells did not respond to treatment with rIL-2.

Analyses Predicting Blastogenic Response of PBLs to Con A, PHA, and the T3 MAb

Table 3 shows regression results for the Con A and PHA blastogenic responses across three concentrations each. Because the findings are similar for both assays, they will be discussed together. For model A, which used age, stage, and days since surgery as the independent variables, the value of R^2_A for Con A ranged from .035 to .054 and was of similar magnitude for PHA, ranging from .022 to .033. Since the number of total T cells available will affect the blastogenesis values, we next added the number of T3-positive cells into the analyses as an additional, independent control variable as shown by the step model AA. Across all concentrations for each mitogen, the value of R^2_{AA} suggested that this variable added variance, yielding the R^2_{AA} values ranging from .105 to .125 for Con A and from .023 to .033 for PHA.

The addition of stress (IES) to the regression for blastogenesis added significant variance, as indicated in model B. All of the R^2 values were statistically significant. Considering the increments in R^2 due to stress (IES), these were significant and ranged from .032 to .061 for Con A and from .047 to .060 for PHA, reflecting the proportion of variance in the blastogenesis accounted for by IES beyond that explained by the control variables. Again, the negative β weights for IES in model B indicated a negative influence of psychologic stress on the blastogenic responses

Table 3. Results of regression analyses for predicting the blastogenic response to concanavalin A (Con A) and phytohemagglutinin A (PHA) across three concentrations each

	Dependent variable: blastogenic response of mitogen					
	Con A			PHA		
	10 μg/mL	5 μg/mL	2.5 μg/mL	10 μg/mL	5 μg/mL	2.5 μg/mL
Model A, R^2_A*	.035	.043	.054	.022	.024	.033
Model AA, R^2_{AA}†	.105	.125	.115	.023	.024	.033
Model B‡						
R^2_B	.166	.174	.147	.083	.074	.080
R^2_{B-AA}§	.061	.049	.032	.060	.050	.047
β_{Stress}‖	−.255	−.229	−.187	−.256	−.234	−.229
$t(df = 103)$¶	−2.668	−2.401	−1.927	−2.521	−2.299	−2.254
P	.010	.018	.058	.014	.024	.026
Model C#						
R^2_C	.053	.065	.053	.070	.054	.052
$t(df = 108)$¶	−2.443	−2.724	−2.443	−2.857	−2.489	−2.441
P	.016	.008	.016	.006	.014	.016

*Model A includes the control predictors of age, stage, and days since surgery for the immune outcome, blastogenesis. The R^2_A is the total variance in blastogenesis explained by these three predictors.

†Model AA includes model A variables plus the control predictor of number of T cells for the immune outcome, blastogenesis. The R^2_{AA} is the total variance in blastogenesis explained by these four predictors.

‡Model B includes model AA control variables plus the stress predictor (i.e., Impact of Event Scale [IES] score) for the immune outcome, blastogenesis. The R^2_B is the total variance in blastogenesis explained by the four control predictors and the stress predictor.

§R^2_{B-AA} is the increment in variance due to stress only (i.e., variance beyond that explained by the control predictors) in predicting the blastogenesis outcome.

‖β_{Stress} is the standardized regression beta (β) for the stress variable in model B. It indicates the magnitude and direction of the influence (negative) of stress on the immune outcome.

¶*df* refers to the degrees of freedom in model B.

#Model C includes stress as the only predictor of the immune outcome, blastogenesis. The R^2_C is the total variance in blastogenesis explained by stress; this model provides the simple association between psychologic stress and immune function.

across concentrations. Moreover, no other predictor in model B had a significant regression coefficient. Finally, results for model C in Table 3 showed a simple association between stress (IES) and the blastogenic response. These correlations were significant for each concentration of Con A and PHA.

Table 4 shows regression results for the proliferative response of T cells to three different dilutions of the T3 MAb. For model A, the control R^2 values were not significant for any dilution. Addition of number of T3-positive cells available as a control increased the variance accounted for as shown by the step model AA. The R^2_{AA} values ranged from .088 to .143. However, increments in R^2 due to the addition of stress (IES), as shown by R^2_{B-AA}, were significant, ranging from .056 to .067. This indicates that about 6% of the variance was accounted for by stress (IES) beyond that explained by the control variables. Once again, no other predictor in model B had a significant regression coefficient. Results for model C again showed the simple, significant association of stress (IES) with the response to the T3 MAb at all dilutions, with R^2_C values of .092 to .102.

Discussion

Any immune response involves a complex cascade of events that occur over time. Studies suggest that the peripheral products of stress can play numerous roles in regulating immunity, and so the effects of stress will, necessarily, be variable. Current research suggests, for example, that the acute stressors, both real stressors [e.g., parachute jumps *(30)*] and artificial stressors [e.g., experimental tasks including speech or math stress *(31)*], are correlated with the mobilization (increase) of NK cells. These changes are thought to be a result of alterations in cell trafficking. In contrast, studies of chronic stressors [e.g., bereavement, caregiving, or divorce *(7,9)*] suggest that stress can have an effect on the ability of NK cells to lyse a target cell, the ability of NK cells to respond to rIFN γ and rIL-2 *in vitro*, and other aspects of the cellular immune response.

Our results suggest that stress, as assessed via a self-report measure of intrusive and avoidant thoughts and behaviors about cancer, was related to a negative effect on NK cell lysis, the ability of NK cells to respond to two cytokines, the blastogenic response of PBLs to two mitogens, and the proliferative response to MAb T-cell receptor. These effects were inhibitory and of similar magnitude (i.e., reliable), both between the assays and within an assay (i.e., across E:T ratios and mitogen concentrations). The analyses controlled for variables that might also be expected to exert short-term or long-term effects on immunity—such as age, stage of disease, and days since surgery—and ruled out other potentially confounding variables (e.g., nutritional status) that might also be influential. These controls reduced the plausibility of alternative, rival hypotheses for these consistent findings.

It is recognized that NK cells mediate natural immunity, but some researchers *(32)* suggest that their role in health generally has been underestimated. For example, there is evidence to suggest that the NK cells participate either directly or indirectly in multiple developmental, regulatory, and communication networks of the immune system. Furthermore, NK cells are efficient effector cells that not only are equipped for cell killing, but also are capable of rapid responses to exogenous or endogenous signals by producing cytokines and other factors involved in interactions between immune and nonimmune cells *(20)*.

The ability to spontaneously lyse a broad range of infected cells or tumor cells is the best known functional attribute of NK cells *(20,22)*. Consistent with previous reports, these data suggest that stress may impair this important process. Our findings highlight the specific effect of cancer stress on immune function, whereas prior data obtained by Levy et al. *(33)* had suggested that women's reports of fatigue were related to lower levels of NK cell lysis. Chronically low levels of NK cell activity occur in patients with cancer, particularly when there are large tumor burdens or disseminated metastases *(32)*. In general, patients with low NK cell activity appear to be at higher risk for infections, to have more prolonged diseases, or to suffer more severe symptoms

Table 4. Results of regression analyses for predicting proliferative response of peripheral blood leukocytes to a monoclonal antibody to T-cell receptor (T3) across three dilutions

	Dependent variable: proliferative response at dilutions		
	128:1	64:1	32:1
Model A, R^2_A*	.026	.052	.064
Model AA, R^2_{AA}†	.088	.104	.143
Model B‡			
R^2_B	.155	.160	.200
R^2_{B-AA}§	.067	.056	.057
β_{Stress}‖	−.273	−.249	−.252
$t(df = 101)$¶	−2.747	−2.514	−2.604
P	.008	.014	.012
Model C#			
R^2_C	.102	.092	.094
$t(df = 101)$¶	−3.452	−3.255	−3.307
P	.002	.002	.002

*Model A includes the control predictors of age, stage, and days since surgery for the immune outcome, proliferative response. The R^2_A is the total variance in proliferation explained by these three predictors.

†Model AA includes model A variables plus the control predictor of number of T cells for the immune outcome, proliferation. The R^2_{AA} is the total variance in proliferation explained by these four predictors.

‡Model B includes model AA control variables plus the stress predictor (i.e., Impact of Event Scale [IES] score) for the immune outcome, proliferation. The R^2_B is the total variance in proliferation explained by the four control predictors and the stress predictor.

§R^2_{B-AA} is the increment in variance due to stress only (i.e., variance beyond that explained by the control predictors) in predicting the proliferation outcome.

‖β_{Stress} is the standardized beta (β) for the stress variable in model B. It indicates the magnitude and direction of the influence (negative) of stress on the immune outcome.

¶*df* refers to the degrees of freedom in model B.

#Model C includes stress as the only predictor of the immune outcome, proliferation. The R^2_C is the total variance in proliferation explained by stress; this model provides the simple association between psychologic stress and immune function.

than patients whose NK cell activity remains normal *(32,34)*.

A variety of biologic response modifiers are known to increase the activation, proliferation, or cytotoxicity of NK cells *(20)*. Among the best known activators of NK cells are IL-2 and IFN γ. Our data show that the physiologic changes associated with psychologic stress inhibited NK cell lysis. Stress also affected the ability of NK cells to respond to rIFN γ, a finding that is consistent with two previous reports involving another life stressor [i.e., caregiving for a spouse with Alzheimer's disease *(9,26)*]. It is interesting that NK cells from 62% of the women did not respond to rIL-2. In subsequent analyses comparing women who did have an rIL-2 response with those who did not, no stress or disease variable differentiated the two groups. Further studies will need to be performed to explore this result, although it is possible that the lack of responsiveness of NK cells to rIL-2 may be due to an overproduction of prostaglandin E_2 by monocytes. It has been suggested that in breast cancer patients prostaglandin E_2 decreases IL-2 production in effector cell populations, resulting in the down-regulation of the expression of the IL-2 receptor on NK cells *(23)*. Follow-up studies will need to pursue and clarify this difference in cytokine responses.

It has been shown that the ability of PBLs to respond to PHA is reduced, in general, in cancer patients *(35)*; this lowered response is related to tumor burden and declines in the ability of PBLs to respond to PHA with disease progression *(36)*. The negative effect of stress on blastogenesis was replicated in this study across two mitogens, PHA and Con A, as well as in the response of T cells to an MAb against the T-cell receptor. These findings are consistent with correlational and experimental studies indicating that stress impairs the blastogenic response of PBLs to mitogens and virus-specific T-cell responses *(8,10,37–39)*. Mitogen-induced proliferation has been used to indicate the immune system's ability to respond to antigens from pathogens. Chronically stressed, but healthy, individuals showing decrements in the cellular immune response (including NK cell lysis and the response of the PBLs to mitogens) subsequently reported a higher incidence of infectious illnesses *(8)*. If this effect is reliable, these data would suggest that cancer patients who experience high levels of stress, lowered levels of responsive T lymphocytes, and decreased NK cell function may be at greater risk for infectious illnesses as they begin adjuvant therapy.

It is interesting that evidence is accumulating to suggest that psychologic and/or behavioral stress reduction interventions may *enhance* certain aspects of the cellular immune response, including NK cell lysis. In an early investigation, Kiecolt-Glaser et al. *(40)* studied 61 healthy adults living in a retirement home. After receiving 1 month of training in progressive muscle relaxation, the subjects showed evidence of a 30% increase in NK cell lysis in comparison with those who received no treatment or only social contact. Fawzy et al. *(41)* studied 61 patients with melanoma and reported that, 6 months after treatment, subjects receiving intervention had significantly higher levels of IFN alfa-augmented NK cell activity than those who received no treatment. These data suggest that, if behavioral interventions can reduce stress and enhance the cellular immune response, then health outcomes might improve.

In conclusion, these data show a down-regulation of different aspects of the cellular immune response associated with the psychologic stress that accompanies the diagnosis and initial surgical treatment of cancer. We note that these study participants are part of a larger effort testing the biobehavioral aspects of stress, immunity, and disease course *(5)*. It will be important to document the longitudinal nature of these findings, and future studies will provide such data. Moreover, half of the women who participated have been randomly assigned to receive a psychologic/behavioral intervention specifically designed to reduce stress, enhance quality of life, and test for the biologic mechanism—such as immune responses—that may mediate any positive effects of stress reduction on health and disease outcomes.

References

(1) Andersen BL, Anderson B, deProsse C. Controlled prospective longitudinal study of women with cancer: II. Psychological outcomes. J Consult Clin Psychol 1989;57:692–7.

(2) Moyer A, Salovey P. Psychosocial sequelae of

breast cancer and its treatment. Ann Behav Med 1996;18:110–25.
(3) Andersen BL. Surviving cancer. Cancer 1994;74(4 Suppl):1484–95.
(4) Shalala DE (Chair). Proceedings: Secretary's Conference to Establish a National Action Plan on Breast Cancer, Dec 14–15, 1993. Bethesda (MD): National Institutes of Health, 1993.
(5) Andersen BL, Kiecolt-Glaser JK, Glaser R. A biobehavioral model of cancer stress and disease course. Am Psychol 1994;49:389–404.
(6) Herbert TB, Cohen S. Depression and immunity: a meta-analytic review. Psychol Bull 1993;113:472–86.
(7) Herbert TB, Cohen S. Stress and immunity in humans: a meta-analytic review. Psychosom Med 1993;55:364–79.
(8) Kiecolt-Glaser JK, Dura JR, Speicher CE, Trask OJ, Glaser R. Spousal caregivers of dementia victims: longitudinal changes in immunity and health. Psychosom Med 1991;53: 345–62.
(9) Esterling BA, Kiecolt-Glaser JK, Bodnar JC, Glaser R. Chronic stress, social support, and persistent alterations in the natural killer cell response to cytokines in older adults. Health Psychol 1994;13:291–8.
(10) Kiecolt-Glaser J., Glaser R, Gravenstein S, Malarkey WB, Sheridan J. Chronic stress alters the immune response to influenza virus vaccine in older adults. Proc Natl Acad Sci U S A 1996;93:3043–7.
(11) Kiecolt-Glaser JK, Marucha PT, Malarkey WB, Mercado AM, Glaser R. Slowing of wound healing by psychological stress. Lancet 1995;346:1194–6.
(12) Levy SM, Herberman RB, Lee J, Whiteside T, Kirkwood J, McFeeley S. Estrogen receptor concentration and social factors as predictors of natural killer cell activity in early-stage breast cancer patients. Confirmation of a model. Nat Immun Cell Growth Regul 1990; 9:313–24.
(13) Fortner JG, Kim DK, Hopkins L, Barrett MK, Pinsky CM, Day NK. Immunologic function in patients with carcinoma of the pancreas. Surg Gynecol Obstet 1980;150:215–8.
(14) Monson JR, Ramsden C, Guillou PJ. Decreased interleukin-2 production in patients with gastrointestinal cancer. Br J Surg 1986; 83:483–6.
(15) Feo Figarella E, Morillo F, Blanca I, Bianco NE. Failure of cell-mediated effector mechanisms in lung cancer. J Natl Cancer Inst 1984; 73:1–6.
(16) Anastasopoulos E, Reclos GJ, Baxevanis CN, Tsilivakos V, Panagiotopoulos N, Fotiou S, et al. Monocyte disorders associated with T cell defects in cancer patients with solid tumors. Anticancer Res 1992;12:489–94.
(17) Steinhauer EH, Doyle AT, Reed J, Kadish AS. Defective natural cytotoxicity in patients with cancer: normal number of effector cells but decreased recycling capacity in patients with advanced disease. J Immunol 1982;129:2255–9.
(18) Jubert AV, Lee ET, Hersh EM, McBride CM. Effects of surgery, anesthesia and intraoperative blood loss on immunocompetence. J Surg Res 1973;15:399–403.
(19) Herberman RB, Ortaldo JR. Natural killer cells: their roles in defenses against disease. Science 1981;241:24–30.
(20) Trinchieri G. Biology of natural killer cells. Adv Immunol 1989;47:187–376.
(21) Hersey P, Edwards A, Honeyman M, McCarthy WH. Low natural-killer-cell activity in familial melanoma patients and their relatives. Br J Cancer 1979;40:113–22.
(22) Whiteside TL, Herberman RB. Characteristics of natural killer cells and lymphocyte-activated killer cells. Immunol Allerg Clin North Am 1990;10:663–704.
(23) Baxevanis CN, Reclos GJ, Gritzapis AD, Dedousis GV, Missitzis I, Papamichail M. Elevated prostaglandin E_2 production by monocytes is responsible for the depressed levels of natural killer and lymphokine-activated killer cell function in patients with breast cancer. Cancer 1993;72:491–501.
(24) Horowitz M, Wilner N, William A. Impact of Event Scale: a measure of subjective stress. Psychosom Med 1979;41:209–18.
(25) Kiecolt-Glaser JK, Glaser R. Methodological issues in behavioral immunology research with humans. Brain Behav Immun 1988;2:67–78.
(26) Esterling BA, Kiecolt-Glaser JK, Glaser R. Psychosocial modulation of cytokine-induced natural killer cell activity in older adults. Psychosom Med 1996;58:264–72.
(27) Gieni RS, Li Y, Hay Glass KT. Comparison of [^{3}H]thymidine incorporation with MTT- and MTS-based bioassays for human and murine IL-2 and IL-4 analysis. Tetrazolium assays provide markedly enhanced sensitivity. J Immunol Methods 1995;187:85–93.
(28) Shobitz B. Steroids and central regulation of immune response. Meth Neuro Sci 1994;22: 510–52.
(29) Cohen J, Cohen P. Applied multiple regression/correlation analysis for the behavioral sciences. Hillsdale (NJ): Erlbaum, 1983.
(30) Schedlowski M, Jacobs R, Stratmann G, Richter S, Hadicke A, Tewes U, et al. Changes in natural killer cells during acute psychological stress. J Clin Immunol 1993;13:119–26.
(31) Uchino BN, Cacioppo JT, Malarkey W, Glaser R. Individual differences in cardiac sympathetic control predict endocrine and immune responses to acute psychological stress. J Pers Soc Psychol 1995;69:736–43.
(32) Whiteside TL, Herberman RB. Role of human natural killer cells in health and disease. Clin Diagn Lab Immunol 1994;1:125–33.
(33) Levy SM, Herberman RB, Maluish AM, Schlien B, Lippman M. Prognostic risk assessment in primary breast cancer by behavioral and immunological parameters. Health Psychol 1985;4:99–113.
(34) Cannon GB, Dean JH, Herbermann RB, Perlin E, Reid J, Miller C, et al. Association of de pressed postoperative lymphoproliferative re sponses to alloantigens with poor prognosis i patients with stage I lung cancer. Int J Cance 1980;25:9–17.
(35) Han T, Takita H. Depression of T lymphocyt response by non-T suppressor cells in lung car cer patients: a possible prognostic value of sup pressor cell activity. Cancer 1979;44:2090–8
(36) Ludwig CU, Hartmann D, Landmann R, Wes M, Rosenfelder G, Stucki D, et al. Unaltere immunocompetence in patients with non disseminated breast cancer at the time of diag nosis. Cancer 1985;55:1673–8.
(37) Baron RS, Cutrona CE, Hicklin D, Russe DW, Lubaroff DM. Social support and immun function among spouses of cancer patients. Pers Soc Psychol 1990;59:344–52.
(38) Bartrop RW, Luckhurst E, Lazarus L, Kilo LG, Penny R. Depressed lymphocyte functio after bereavement. Lancet 1977;1:834–6.
(39) Locke SE, Kraus L, Leserman J, Hurst MW Heisel JS, Williams RM. Life change stress psychiatric symptoms, and natural killer cel activity. Psychosom Med 1984;46:441–53.
(40) Kiecolt-Glaser JK, Glaser R, Williger D, Stou J, Messick G, Sheppard S, et al. Psychosocia enhancement of immunocompetence in a geri atric population. Health Psychol 1985;4 25–41.
(41) Fawzy FI, Kemeny ME, Fawzy NW, Elashof R, Morton D, Cousins N, et al. A structured psychiatric intervention for cancer patients. II Changes over time in immunological mea sures. Arch Gen Psychiatry 1990;47:729–35.

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